ACTIVE IMMUNITY PRODUCED BY SO-CALLED BALANCED OR NEUTRAL MIXTURES OF DIPHTHERIA TOXIN AND ANTITOXIN.[1]

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In a former publication[2] I briefly called attention to the fact that an active immunity may be induced in guinea-pigs by mixtures of diphtheria toxin and antitoxin which, after subcutaneous injection, produce no local lesion recognizable during the life of the animal, no general disturbances indicated by loss in weight, and no paralysis. This acquired immunity remains in evidence at least two years.

This phenomenon is of both theoretical and practical importance. As the facts will show, it does not harmonize fully with current theories of the relation between toxins and antitoxins in mixtures. From the practical standpoint it offers a promising field for investigations in the active immunization of the human subject. If the latter reacts as does the guinea-pig, it should be an easy matter to confer a relatively high degree of active immunity, lasting at least several years, without any appreciable disturbances of health. The experiments recorded and tabulated below were planned with these two lines of inquiry in mind, and offer a basis for further investigations upon a very important subject.

METHOD.

The interpretations and inferences drawn from the results of this series of experiments are based upon the fact observed by a number

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[2] The degree and duration of passive immunity to diphtheria toxin transmitted by immunized female guinea-pigs to their immediate offspring. *Jour. of Med. Research*, 1907, xvi, 359.

of authors and by the writer, that an actively immunized female parent may transmit antibodies to the immediate young, who, receiving the immunity passively, soon lose it again. If active immunity of the mother reveals itself as passive immunity in the immediate offspring, the degree of passive immunity in the latter may be taken as a measure, relative only, to be sure, of the active immunity of the mother. In the series of experiments described below this principle was assumed to be true, and made use of throughout. Young female guinea-pigs received subcutaneously mixtures of diphtheria toxin and antitoxin, and the degree of passive immunity of their offspring was determined as a relative measure of the active immunity of the female parent.

Two other facts were fairly well established in the earlier paper quoted. Guinea-pigs receiving one, two or three doses of antitoxin subcutaneously in doses of three to five cubic centimeters did not transmit any immunity to their offspring several months later. Guinea-pigs which had recovered from a nearly fatal dose of toxin only did not acquire thereby enough active immunity to raise the normal resistance of their offspring appreciably. The first fact agrees with current knowledge that passive immunity is not transmitted to offspring unless perhaps the antibodies are injected during pregnancy. The second fact shows that there must be a certain degree of active immunity possessed by the mother to make any impression on the resistance of the offspring.[3]

The method which was followed throughout may be briefly described here to avoid needless repetition. Young female guinea-pigs whose genealogy was known were used for immunization. As a rule two or three of the same litter were chosen for the same experiment, to eliminate family differences. They received subcutaneously mixtures of diphtheria toxin and antitoxin of varying proportions. Each animal received but one dose before it was bred.

[3] Anderson (*Pub. Health and M. H. Service, Hygienic Laboratory Bull.*, 1906, No. 30) observed, independently of the writer, the absence of any transmitted immunity following the injection of toxin alone. He observed a certain degree of immunity in the young following the treatment of the mother with antitoxin alone. As all five injections, amounting to 4250 units, were made during pregnancy, and as the results of the tests of the second and third litters were irregular, the immunity of the mother was probably passive, and his data, therefore, do not invalidate the position here taken—that antitoxin alone does not confer active immunity.

The first litter was born three or more months after the injection of the mixture into the parent.

To test the resistance of the young, two procedures were available. One was to inject pure toxin and thereby determine the increase in the minimum fatal dose. The other was to inject mixtures of toxin and one antitoxic unit, and determine the increase of toxin in the L_+ dose, *i. e.*, the dose just necessary to kill the guinea-pig in the presence of one antitoxic unit. In the present work the second method was used exclusively.[4]

Since the passive immunity of the offspring gradually declines and practically disappears at the end of three months, the degree of immunity was determined as nearly as possible when the young were thirty days old. This rule was departed from in certain instances and allowance was made for it in estimating the resistance. In the fall and winter the growth of the young in weight is very slow, as compared with the spring and summer. Hence, there is much variation at this age in the weight of the animals undergoing the test, the extremes being 162 and 336 grams. The low weight did not, however, appear to seriously influence the resistance of the animals as shown by the immunity of litters of low and high weight belonging to the same mother.

The other method available for determining the active immunity of the treated mother was to test the antitoxic content of her blood[5] directly. For several reasons this method was not chosen. It would have been desirable to establish some relation between the antitoxic content of the mother's blood and the resistance of the offspring. This we hope to do in the near future. In the meantime some conception of the resistance of the mother was obtained by a final injection of a toxin-antitoxin mixture when the experiment had been concluded.[6]

[4] Repeated tests had shown that the minimum fatal dose is relatively high in the passively immune offspring. The precise numerical relation of this rise to that of the L_+ dose has not yet been studied.

[5] In the paper cited, one animal was subjected to this test which had received a nearly neutral mixture of toxin and antitoxin about one and one-half years before. The blood serum contained approximately $\frac{1}{4}$ unit per cubic centimeter.

[6] All the tests were made with one lot of toxin under toluol whose L_+ dose (minimum fatal dose in the presence of one antitoxic unit intimately mixed

In the course of the investigation several distinct questions presented themselves for solution, all of which are partly answered in the tabulated results given below. They may be briefly stated as follows:

1. What is the smallest amount of toxin added to one unit of antitoxin which confers enough active immunity upon a female so that it may be detected in the offspring three or more months later?

2. Is the immunity conferred by a non-neutralized mixture, *i. e.*, one which still produces local lesions, greater than that produced by a neutralized mixture, which produces no local lesion or paralysis?

3. Can the immunity produced by a single antitoxic unit plus a given amount of toxin be heightened by multiples of this mixture?

4. Does a toxin-antitoxin mixture confer as much immunity after standing several days as it does immediately after preparation?

5. Are there noteworthy differences, as regards the degree of acquired active immunity, following the same treatment among litters from different families, and among individuals of the same litter?

In the tabulated results given below, these five topics are not sharply separated, for some of the experiments throw light on several at the same time. They will not, therefore, be taken up in the order given here, but the experiments will be described more or less chronologically.

Before passing to the experiments proper, it is desirable to call attention to two minor questions bearing directly on them. One is the L_+ dose of adult guinea-pigs, since nearly all of the mothers after completion of the breeding experiments herein described were tested finally to determine their own resistance. In Table I three adult guinea-pigs, presumably of normal susceptibility, were tested. The L_+ dose for young pigs at this time was 0.21 c.c. The table

with it before injection) was 0.21 to 0.235 c.c. during the period of the entire experiment, lasting two years. The L_0 dose was 0.17 c.c. at the beginning of these experiments. At the conclusion of the last tests the L_0 dose was still 0.17 c.c.; 0.18 c.c. produced slight transient œdema and 0.19 c.c., transient induration, roughening of skin and slight loss of hair. Unless statement is made to the contrary, the toxin-antitoxin mixture was injected about 15 minutes after preparation. For the sake of uniformity the standard serum, issued by the Institute for Experimental Therapy under the direction of Professor Ehrlich, and used in the earlier experiments, was continued in the present series.

shows that for old adults it is about 0.30 c.c. This figure should be borne in mind in studying the resistances of the treated mothers.

TABLE I.

Adult Controls.

Designation.	Sex.	Weight.	Age.	Dose injected in c.c.	Result.	Remarks.
3053	Female.	580	4 years and 7 months.	.30 toxin + 1 unit antitoxin.	Dies in 3½ days.	Received 2 doses of antitoxin, about 5 c.c. each, early in life.
8093	Male.	672	5 months and 6 days.	.35 toxin + 1 unit antitoxin.	Dies in 2¾ days.	
4341	Female.	965	2 years and 2 months.	.30 toxin + 1 unit antitoxin.	Dies in 3½ days.	Received some antitoxin early in life.

The second matter relates to passive immunity transmitted, during the period of lactation, in the milk. The influence of lactation upon the immunity of the offspring in mice, so strikingly demonstrated in Ehrlich's now celebrated experiments with ricin and abrin, had not been satisfactorily defined in the case of guinea-pigs actively immunized with diphtheria toxin. In the series of experiments here described it became desirable to determine how far lactation was a factor. In Table II is given a crucial experiment, which goes to show that lactation plays no appreciable part in the passive immunity of the young, and that it may be neglected as a factor. No. 3896, a normal, untreated female, nursed the offspring of a highly immunized female, No. 4977, from the first day to the time

TABLE II.

Mother.						Offspring.						Remarks.
Designation.	Estimated L_+ Dose When 30 Days Old, in c.c.	Dose Injected in c.c.	Result.	Second Dose Injected in c.c. (Period Between First and Second Dose).	Result.	Period Between Treatment of Mother and Birth of Litter.	Age in Days.	Weight in Grams.	Dose of Toxin + 1 Antitoxin Unit in c.c.	Result.	Estimated L_+ Dose.	
3896	.215	—	—	—	—	—	31	210	.21	Dies in 5½ days.		This litter was nursed by the highly immune mother No. 4977 from day of birth to day of test.
							36	213	.22	Dies in 2⅙ days.	.21	
4977	"	.17 toxin + 1 unit antitoxin.	No lesion.	.40 toxin + 1 unit antitoxin (5 months and 27 days).	Trace of induration.	7 months and 1 month.	31	332	.30	No lesion.		This litter was nursed by the normal mother No. 3896 from the day after birth until the day of test.
							31	351	.35	Transient induration.	.35 + +	

of testing, thirty-one days after birth. Both young showed a very high degree of resistance. On the other hand, the offspring of No. 3896, nursed by the highly immune mother, No. 4977, from the day after birth until the day of the test, showed only the normal resistance.

ANALYSIS OF THE EXPERIMENTAL DATA.

On Table III are given all the facts pertaining to four female guinea-pigs treated with unsaturated and supersaturated mixtures of toxin and antitoxin. The treated guinea-pigs belong to two litters, A and B, respectively. They possessed, when treated, a certain degree of passive immunity inherited from treated mothers. This immunity is noted in Column 2 of the table. Much of this had probably passed away in Litter A, because the animals were treated when seventy-five days old.[7] The first of each pair received a mixture in which the toxin was in excess, for in each case an ulcer was produced. The second of each pair received a mixture containing two antitoxic units and, therefore, below the L_0 dose (.17 c.c.) for normal pigs, and probably considerably below this for these passively immune pigs. There was, therefore, no local or general effect produced by the injection.

Two litters each of the first, second and third, and three of the fourth animal were tested. These were born from three and a half to ten months after the single treatment of the mother.

In the table are given the age of the offspring, their weight, the dose injected and the result. From these data the L_+ dose for each litter is estimated and given in the eleventh column of the tables. This L_+ dose is approximate only. When it is a little higher than the figure given a single + is added. Where it is considerably higher two ++ are added. The effect of adding an excess of antitoxin to the toxin is well brought out in the passive immunity transmitted to the young. Bearing in mind that the L_+ dose of the toxin used was at this time 0.215 c.c. for normal animals, we observe that it is very high in the offspring of the first of each pair, and that it is but slightly above normal in offspring of the

[7] When the age is not given, the future mothers were treated when about 30 days old.

second. It is, in fact, nearly twice as high in the offspring of the first. It is of interest to note, however, that even a toxin to which a relatively large amount of antitoxin has been added and which is injected into guinea-pigs possessing some passive immunity still produces enough immunity just to appear in the young months later, as shown in the slight elevation of the L_+ dose.

TABLE III.

Mother.						Offspring.						
Designation.	Estimated L_+ Dose When 30 Days Old, in c.c.	Dose Injected in c.c. (Age of Pig).	Result.	Second Dose Injected (Period Between First and Second Dose).	Result.	Period Between Treatment of Mother and Birth of Litter.	Age in Days.	Weight in Grams.	Dose of Toxin + 1 Antitoxin Unit in c.c.	Result.	Estimated L_+ Dose.	Remarks.
								First litter				
4684	.35	.21 toxin + 1 unit antitoxin (75 days).	Small ulcer.	.45 toxin + 1 unit antitoxin (9 months and 11 days.)	No lesion.	4 months and 8 days.	24	285	.28	No lesion.		
							24	282	.24	ditto.	.28++	The L_+ dose for a normal guinea-pig of 250 grams = .215 c.c. toxin
								Second litter				
A						6 months and 15 days.	36	256	.38	Large ulcer.	.45+	
							40	265	.45	Dies in 5 days.		
								First litter				
4685	ditto.	.21 toxin + 2 units antitoxin (75 days).	No lesion.	.25 toxin + 1 unit antitoxin (9 months and 11 days).	Small ulcer.	3 months and 16 days.	29	265	.22	Moderate ulcer.		Toxin-antitoxin mixtures about 15 minutes old when injected.
							35	298	.24	Dies in 5 days.	.24	
								Second litter				
						6 months and 24 days.	40	301	.23	Dies in 2½ days.		
							36	272	.25	Dies in 2 days.	.23	
								First litter				
4788	.40	.27 toxin + 1 unit antitoxin (37 days).	Ulcer.	.70 toxin + 1 unit antitoxin (10 months and 23 days).	Small ulcer.	6 months and 22 days.	22	253	.30	Transient induration.	.35++	
							25	268	.35	ditto.		
								Second litter				
							31	192	.45	Dies in 2½ days.		
B							31	205	.50×	Dies in 4 days		× Lost ¼ of mixture during injection.
							31	206	.55[c]	Dies in 1½ days.	.50±	[c] Injection in part intraperitoneal.
							31	220	.60	Dies in 2½ days.		
								First litter				
4789	ditto.	27 toxin + 2 units antitoxin (37 days).	No lesion.	.50 toxin† 1 unit antitoxin (10 months and 23 days).	Dies in 2½ days +	4 months and 26 days.	27	243	.215	Small ulcer.		
							27	281	.25	Large ulcer.	.28+	† Injection partly intraperitoneal.
							31	293	.28	Large ulcer.		
								Second litter				
						7 months and 4 days.	35	232	.25	Dies in 2½ days.		
							35	291	.30	Dies in 1½ days.	.24±	
								Third litter				
						10 months and 6 days.	34	264	.25	Dies in 2½ days.	.24	
							34	271	.30	Dies in 1½ days.		

The active immunity of the mothers was finally tested from nine to ten months after the early dose was administered. The difference

in the result between the members of each pair is shown in Columns 5 and 6, although that difference is not accurately defined by the test. The active immunity of the mother runs parallel to the passive immunity of the offspring.

On Table IV further experiments on the immunizing effects of balanced or L_0 doses are recorded. The three females of Litter C were of normal parentage, *i. e.,* not passively immune, as indicated in Column 2. The first received a little less than the L_0 dose of

TABLE IV.

Mother.						Offspring.						Remarks.
Designation.	Estimated L_+ Dose when 30 Days Old, in c.c.	Dose Injected in c.c.	Result.	Second Dose Injected in c.c. (Period Between First and Second Dose).	Result.	Period Between Treatment of Mother and Birth of Litter.	Age in Days.	Weight in Grams.	Dose of Toxin + 1 Antitoxin Unit.	Result.	Estimated L_+ Dose.	
4875	.215	.15 toxin + 1 unit antitoxin.	No lesion.	.45 toxin + 1 unit antitoxin (8 months).	Trans. oedema	4 months and 4 days.	First litter					L_+ dose for normal guinea-pig of 250 grams = 0.215 c.c.
							28	278	.21	No lesion.		
							28	259	.25	Transient swelling.		
							31	246	.30	Large ulcer	.35	
							35	271	.35	Dies in 4½ days.		
C						6⅓ mos.	Second litter					
							30	243	.35	Dies in 3½ days.		
							30	215	.35	Large ulcer.	.35	
4876	ditto.	.27 toxin + 2 units.	ditto.	ditto.	ditto.	3 months and 27 days	First litter					
							28	278	.215	No lesion.		
							28	272	.27	Small ulcer.	.30+	
							32	315	.30	Large ulcer.		
						7 months and 3 days.	Second litter					
							26	238	.35	Dies in 3 days.		
							26	217	.40	ditto	.35	
4877	d tto.	.38 toxin + 3 units antitoxin.	ditto.	ditto.	ditto.	4 months and 1 day.	First litter					
							28	255	.25	No lesion.		
							34	282	.30	Moderate ulcer		
							34	283	.35	Dies in 2½ days.	35	
							38	320	.35	Large ulcer.		
						7 months and 9 days.	Second litter					
							20	207	.35	Moderate ulcer.		
							20	211	.40	Large induration.	.35+	

toxin plus 1 unit antitoxin with no local or general reaction. The second received nearly double this dose of toxin, plus 2 units antitoxin; the third nearly triple the dose of the first plus 3 units antitoxin without any reaction. Taking the L_0 dose as 100 per cent., they received respectively 12, 20 and 24 per cent. less toxin than the L_0 dose. Two litters of each treated female were tested. The

results show a relatively high passive immunity of the young, practically the same in all. Taking the normal L_+ dose as 100 per cent., the actual L_+ dose was 35/21.5 or 163 per cent. of the normal.

The experiment does not inform us just what the effect of doubling and tripling the toxin-antitoxin dose is, as the actual doses of toxin were a trifle less for the second and third animal, but it does demonstrate the high degree of immunity transmitted by apparently balanced mixtures which produce no recognizable effect after injections. The relatively high active immunity of the three mothers is shown in Columns 5 and 6, and indicates a doubling of the L_+ dose.

The experiment reported on Table V is similar to that of Table IV, with certain differences to be noted. The three females of Litter D were born of an actively immunized mother, and their L_+ dose is estimated at .60 or 60/21 of the normal at the age of thirty days. They received doses in which there was enough toxin in excess for them to produce local ulcers. The doses are graded nearly as in Table IV, the second and third animals receiving respectively nearly two and three times the toxin-antitoxin mixture of the first. As a result the active immunity of the mothers after ten to twelve months is very high, for the L_+ dose is probably three times the normal for pigs of their weight (Columns 5 and 6). Their resistance is not the same, however, but increases from first to third. The same is true of the passive immunity of the offspring of which two litters from each mother were tested. The estimated L_+ dose rises from first to third group, and is very high in the third, corresponding to 63/21, or to about three times the normal.

A comparison of Tables IV and V shows that the unbalanced mixtures, or such as produce local lesions, give, as might have been anticipated, a much higher degree of immunity than those equivalent to or below the L_0 dose.

The experiment recorded on Table VI is somewhat like that of Table V. It was made to determine whether the three offspring of the same mother would acquire the same degree of immunity after treatment with the same dose. The litter was born of an immunized mother, and the degree of passive immunity at thirty days was estimated at .33 or 33/21 of the normal. At the age of fifty-eight

days much of this passive immunity must have disappeared, for in each the normal L_+ dose produced a larger ulcer. Tested again over nine months later the first was slightly less resistant than the others.

Only one litter of each was tested. The young of the second

TABLE V.

Mother.						Offspring.						Remarks.
Designation.	Estimated L_+ Dose when 30 Days Old, in c.c.	Dose Injected in c.c. (Age of Pig).	Result.	Second Dose Injected in c.c (Period Between First and Second Dose).	Result.	Period Between Treatment of Mother and Birth of Litter.	Age in Days.	Weight in Grams	Dose of Toxin + 1 Antitoxin Unit in c.c.	Result.	Estimated L_+ Dose.	
								First litter				
4799	.60	.28 toxin + 1 unit antitoxin (42 days).	Small ulcers.	.80 toxin + 1 unit (12½ months).	Large ulcer.	5 months and 19 days	29	290	.25	No lesion.		L_+ dose for a normal guinea-pig of 250 grams = 0 21 c.c.
							32	297	.30	Trans. induration.	.35++	
							32	287	.35	ditto.		
								Second litter				
						11 months and 7 days	33	173	.40	Large ulcer.		
							33	106	.45	ditto.	.50	
							33	199	.50	Dies in 3½ days.		
								First litter				
4801 D	ditto.	.50 toxin + 2 units antitoxin (42 days).	Large ulcer.	.80 toxin + 1 unit antitoxin (10 months).	Moderate induration.	6½ mos	28	254	.30	No lesion.		
							31	275	37	ditto.	.50+	
							35	265	.45	Small ulcer.		
							39	270	.50	ditto.		
								Second litter				
						9 months	30	206	.55	Dies in 7 days.		
							30	230	.60	Large ulcer.	.60	
							30	265	.65	Dies in 6½ days.		
								First litter				
4800	ditto.	.75 toxin + 3 units antitoxin (42 days).	Large ulcer.	.80 toxin + 1 unit antitoxin (13 months).	Slight transient oedema	5 months	32	267	.45	Trans induration.		
							32	274	.55	ditto.		
							36	240	.60	Small ulcer.	.65+	
							36	268	.65	ditto.		
								Second litter				
						12 months	33	178	.50	Trans. induration.		
							33	188	.55	Induration and loss of hair.	.65+	
							33	198	.60	Superficial necrosis.		

were slightly more resistant, but this may have been due in part at least to their greater weight when tested.

On the whole there is little difference between these identically treated animals, and it remains uncertain whether this slight difference is within the margin of error of the method or not. It was hoped that this experiment might reveal certain differences traceable to the father. For if the latter exerts any influence upon his

offspring it must be in transmitting a greater or less capacity for the production of antibodies under treatment. That he does not transmit any antibodies or passive immunity has been abundantly shown.

TABLE VI.

Mother.						Offspring.					Remarks.
Designation.	Estimated L_+ Dose when 30 Days Old, in c.c.	Dose Injected in c.c. (Age of Pig).	Result.	Second Dose Injected in c.c. (Period Between First and Second Dose).	Result.	Period Between Treatment of Mother and Birth of Litter.	Age in Days.	Weight in Grams.	Dose of Toxin + 1 Antitoxin Unit in c.c.	Result.	Estimated L_+ Dose.	
4865 E	.33	.21 toxin + 1 unit antitoxin (58 days).	Large ulcer.	.60 toxin + 1 unit antitoxin (9 mos. and 11 days).	Small ulcer.	8 mos. and 13 days.	29	162	.30	Small ulcer.	.35+	
							34	163	.35	Moderate ulcer.		
4867	ditto.	ditto.	ditto.	ditto.	Trace of indura.	8 months.	28	248	.30	No lesion.	.40±	
							33	207	.40	Large ulcer.		
4868	ditto.	ditto.	ditto.	ditto.	ditto.	8 mos. and 11 days.	31	175	.35	Small ulcer.		
							36	176	.35	Moderate ulcer.	.35+	

The experiments detailed on Tables VII and VIII have both a theoretical and a practical bearing. Leaving aside the former, we may consider briefly the latter before referring to the tabulated results.

If the injection of a balanced mixture of toxin and antitoxin leads to an active immunity, what is the effect if such a mixture has stood for several days? If such mixtures should be found applicable to the active immunization of human beings, they would be of little value if they deteriorated very rapidly. Hence the tests were made with this point in view. The preliminary experiment made on three females of Litter F is given on Table VII. These animals were of normal susceptibility. They received a toxin-antitoxin mixture, which produced no local lesion.

The first received the mixture fifteen minutes after preparation. The second received a similar mixture after it had stood at 35° C. for one hour, and at room temperature for five and one-half hours. The third received a like mixture which had been kept at 35° C. for one hour, and at room temperature for twenty-two hours. Unfortunately this animal did not breed, but the final test on the three about six months later showed a trifling difference in resist-

ance among the three and in favor of the first animal. The offspring of the second, however, possessed a higher passive immunity than those of the first, proving that the toxin-antitoxin mixture had at least remained unharmed, if it had not been actually improved for immunizing purposes by the incubation.

To imitate conditions in practice more closely the tests recorded on Table VIII were made. Three females belonging to each of two litters, G and H, were treated with toxin-antitoxin mixtures,

TABLE VII.

Mother.						Offspring.						Remarks.
Designation.	Estimated L_+ Dose when 30 Days Old, in c.c.	Dose Injected in c.c.	Result.	Second Dose Injected in c.c. (Period Between First and Second Dose).	Result.	Period Between Treatment of Mother and Birth of Litter.	Age in Days.	Weight in Grams.	Dose of Toxin + 1 Antitoxin Unit	Result.	Estimated L_+ Dose.	
4977	.215	.17 toxin + 1 unit antitoxin.	No lesion.	.40 toxin + 1 unit antitoxin (5 mos. and 27 days).	Trace of induration.	4 mos.	33	275	.25	Transient swelling.		Toxin-antitoxin injected into No. 4977 15 minutes after mixing.
							33	294	.30	Large ulcer.	.30+	
F 4978	ditto.	ditto.	ditto.	ditto.	Slight induration.	4 months and 3 days.	31	247	.25	Slight induration.		Toxin-antitoxin mixture injected into No. 4978 after having been kept at 35° C. for 1 hour and at room temperature for 5½ hours.
							31	241	.30	Moderate ulcer.	.35+	
							37	245	.30	Large ulcer.		
							37	246	.35	ditto.		
4976	ditto.	ditto.	ditto.	ditto.	Moderate induration.	Did not	b	reed				Mixture injected into No. 4976 after it had been kept at 35°C. for 1 hour and at room temperature for 22 hours.

which had stood respectively fifteen minutes, two days and five days in a fluctuating room temperature, protected from diffuse light. In the dose given to Litter G the amount of toxin was 0.12 c.c. or about 12/23 of the L_+ dose. That given to Litter H was 0.15 c.c. or 15/23 of the L_+ dose, and it was somewhat below the L_0 dose. After the injection the animals were examined and weighed daily for several weeks, but no appreciable effect, local or otherwise, following the injection was noticed. One of the second litter (H) died before any young were born. Two litters from each of the remaining five mothers, as well as the mothers themselves, were tested to determine any existing immunity.

Several facts are clearly brought out in this table. The toxin-

antitoxin mixture, even when the toxin content is well below the L_0 dose, produces an active immunity in guinea-pigs after the mixture has been kept five days at 50°–70° F. The toxin in these mixtures

TABLE VIII.

Mother.						Offspring.						
Designation.	Estimated L_+ Dose when 30 Days Old, in c.c.	Dose Injected in c.c.	Result.	Second Dose Injected in c.c. (Period Between First and Second Dose).	Result.	Period Between Treatment of Mother and Birth of Litter.	Age in Days.	Weight in Grams	Dose of Toxin + 1 Antitoxin Unit in c.c.	Result.	Estimated L_+ Dose.	Remarks.
8090	.23	.12 toxin + 1 unit antitoxin.	No lesion.	.40 toxin + 1 unit antitoxin (8 mos. and 17 days).	No lesion.	4 months and 10 days.		First litter				
							26	260	.23	Transient induration.	.23++	Toxin - antitoxin injected into No. 8090 15 minutes after mixing.
								Second litter				
						7 months and 4 days.	30	336	.235	Slight trans. induration.		
							30	312	.26	Superficial necrosis.	.29+	
G							30	310	.29	Moderate ulcer.		
								First litter				
8088	ditto.	ditto.	ditto.	.45 toxin + 1 unit antitoxin (9½ months).	Trans. oedema	5 months and 4 days.	32	205	.26	Transient induration.		Toxin - antitoxin injected into No. 8088 after mixture had stood 48 hours at 50° –70° F.
							32	203	.28	ditto.	.28++	
								Second litter				
						8½ mos.	29	265	.26	ditto.		
							29	273	.28	ditto.	.32++	
							29	295	.32	Induration.		
								First litter				
8089	ditto.	ditto.	ditto.	.45 toxin + 1 unit (9 months).	Small ulcer.	4 months and 21 days.	25	220	.235	Transient induration.		Toxin - antitoxin injected into No. 8089 after mixture had stood at 50°–70° F. for 5 days.
							32	255	.25	ditto.	.27+	
							32	200	.27	Moderate ulcer.		
								Second litter				
						7 months and 25 days.	31	178	.24	Small ulcer.		
							31	202	.27	Moderate ulcer.	.30+	
							31	208	.30	Large ulcer.		
8080	ditto.	.15 toxin + 1 unit antitoxin.	ditto.		Dies of	infectious disease						Toxin - antitoxin injected into No. 8080 15 minutes after mixing.
								First litter				
8078	ditto.	ditto.	ditto.	.45 toxin + 1 unit antitoxin (9 months).	Large ulcer.	5⅔ mos.	30	311	.25	Large swelling.		Toxin - antitoxin injected into No. 8078 after mixture had stood 48 hours at 50° –70° F.
							30	305	.28	Large ulcer	.28+	
H								Second litter				
						8 months.	32	243	.25	Superficial necrosis.		
							32	241	.275	Large ulcer.	.30+	
							32	238	.30	Very large ulcer.		
								First litter				
8079	ditto.	ditto.	ditto.	.45 toxin + 1 unit antitoxin (8 months and 25 days).	Very large ulcer.	5½ mos.	32	225	.32	Dies in 2½ days.		Toxin - antitoxin injected into No. 8079 after mixture had stood 5 days at 50°–70° F.
							32	208	.27	ditto.	.26	
							37	260	.235	Large ulcer.		
								Second litter				
						7 months and 23 days.	34	270	.24	ditto.		
							34	263	.26	Dies in 4½ days	.26	
							34	263	.28	Dies in 2½ days.		

does not completely disappear as such, but exercises some influence in the body of the guinea-pig, although the latter is not injuriously affected by the immunization.[8] In the second place the active immunity of the mother and the passive immunity of the offspring is slightly higher, under the influence of the two-day mixture than under either the fifteen-minute or the five-day mixture.

This table, furthermore, clearly shows differences existing among families of guinea-pigs in the capacity to produce antibodies, *i. e.*, to become immune. Thus, Litter H received 25 per cent. more toxin in the toxin-antitoxin mixture than Litter G, yet the two females of Litter H were less actively immune and their young showed a lower degree of passive resistance than those of Litter G.

INTERPRETATIONS AND CONCLUSIONS.

The foregoing and earlier data taken together demonstrate that an active immunity lasting several years can be produced in guinea-pigs, by the injection of toxin-antitoxin mixtures which have no recognizable harmful effect either immediate or remote. They also show, what might have been anticipated, that under the same conditions mixtures which produce local lesions and which, therefore, contain an excess of toxin produce a much higher degree of immunity than the neutral mixtures, and that an excess of antitoxin reduces the possibility of producing an active immunity, and may extinguish it altogether. There is, therefore, a certain definite relation between the components of the mixture and the degree of immunity producible. Furthermore, toxin-antitoxin mixtures do not change materially within five days at room temperature. They are apparently more efficacious at the end of forty-eight hours than immediately after preparation. The experiments finally prove that a relatively high degree of active immunity can be induced by a harmless procedure, whereas the use of toxin alone leading to very severe local lesions is incapable of producing more than an insignificant protection.

The method, therefore, invites further tests in regard to its ulti-

[8] Experiments not yet completed indicate that some immunity is produced by toxin-antitoxin mixtures kept four days at room temperature, even when the toxin dose is but one-half of the L_0 dose.

mate applicability to the human being. Unless the subcutis of the guinea-pig reacts to toxin-antitoxin mixtures in a manner peculiar to itself, a practical, easily controlled method for active immunization can be worked out which should afford a larger protection than the serum alone and avoid the complications associated with horse serum. That proportion of toxin and antitoxin which would produce the highest desirable immunity consistent with the least discomfort would have to be carefully worked out for the human subject. From the nature of the immunity induced it is obvious, however, that such a method of immunization cannot take the place of a large dose of antitoxin in exposed individuals who must be protected at once. It would be applicable only as a general protective measure without reference to any immediate danger, since it would take several weeks, perhaps longer, to perfect the attainable immunity.

Passing to the theoretical aspects of the facts observed, we find no publications bearing directly upon the subject before us. Madsen[9] has, however, approached it very closely in his experiments on the immunization of animals with mixtures not fully balanced, or, in other words, in which the "toxones" were still free. He found that the injection of such mixtures in rabbits, goats and horses produces an active immunity. He makes the significant remark that perhaps in the immunizing capacity we may possess the keenest reagent for a poison which is not able to exert any toxic action in the body. This is fully borne out by the experiments described, for in these we pass beyond the visible spectrum, so to speak, of the toxin-antitoxin effects, and we are able to recognize toxic action only by the lasting immunizing effects.

Another publication which touches upon some phases of the same problem is that of Morgenroth on the union between toxin and antitoxin.[10] Morgenroth brought out the fact that a given toxin-antitoxin mixture is more toxic when injected directly into the circulation than when injected under the skin. Thus, an L_+ dose of 0.78 c.c. toxin + one unit antitoxin applied subcutaneously was of the same toxicity as 0.68 c.c. toxin + one unit antitoxin injected

[9] *Zeit. für Hygiene u. Infectionskrankheiten,* 1901, xxxvii, 251.
[10] *Zeit. für Hygiene u. Infectionskrankheiten,* 1904, xlviii, 177.

into the circulation. When the mixture had stood twenty-four hours this (L_+) dose was still 0.78 c.c. subcutaneously, but it had risen to 0.74 c.c. when introduced by the intracardiac route. The author makes two deductions from these results. He assumes that the velocity of reaction between toxin and antitoxin is slow, and that the union is not completed until the mixture has stood twenty-four hours. Hence, the L_+ dose of toxin injected into the blood is higher after twenty-four hours than immediately after mixing the toxin and antitoxin. He furthermore explains the fact that the subcutaneous L_+ dose remains the same whether the mixture is injected at once or after twenty-four hours, by assuming that in the subcutis of the guinea-pig there is a catalytic acceleration of the union of toxin and antitoxin.

In view of the writer's results it seems that not only immediately, but four to five days after the preparation of the mixture of toxin and antitoxin, there are still toxic substances available for the production of immunity in the body of the guinea-pig, when the dose of toxin in the mixture is far below the L_0 or neutral level. These toxins may be free, either because uncombined *in vitro,* or else because the mixture is partially dissociated *in vivo,* or there may be a third possibility. It is obvious that Morgenroth's investigations, however extensive and thorough, have not exhausted the subject, for both these inferences are incompatible with his. Perhaps his recent important studies on the recovery of toxin from its combination with antitoxin with weak acids may throw more light on this subject.[11]

The only conclusion which we may safely draw at this time is that the toxin-antitoxin mixture produces two sets of effects, essentially identical, however. One is visible, as injury (œdema, loss of hair, superficial and deep necrosis of skin, paralysis and death), and corresponds to the toxin spectrum of Ehrlich. The other is invisible and manifests itself only in degrees of active immunity. At what ratio of toxin to antitoxin in the mixture active immunity is no longer produced will vary somewhat with the guinea-pig used, but it is evident that traces of immunity are still transmitted to the young when the amount of toxin approaches half the L_0 dose.

[11] *Virchow's Archiv.*, 1907, cxc, 371.

THE HISTOLOGICAL CHANGES IN NERVE CELLS DUE TO TOTAL TEMPORARY ANÆMIA OF THE CENTRAL NERVOUS SYSTEM.[1]

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Plate IV.

In the course of some experiments on the resuscitation of the central nervous system, the question arose as to whether it would be possible to determine from the histological appearance of the cells, after being subjected to anæmia for a given period, which of them might recover under proper conditions, and which of them were injured beyond the possibility of recovery. Again, in cases of recovery, it was thought that there might be some relation between the degree of functional return and the morphological appearances of the nerve cells. The present paper attempts a partial answer to these questions.

Previous Histological Work.—The classical experiment of Stenson and Swammerdam—ligation of the abdominal aorta for varying periods of time—first described by these two investigators and later by Haller, has been repeated by many workers in order to determine the histological changes resulting from interference with the blood supply of the spinal cord.

A review of the literature up to the time of publication of their paper is given by de Buck and de Moor (1). Most of the work was done on the spinal cord. Artificial anæmia was induced by Stenson's modified method, *i. e.*, passing a curved needle and thread around the spinal column at about the fourth lumbar vertebra, thus compressing the aorta against it (Ehrlich and Brieger (2), Singer (3), Spronck (4), Herter (5), Singer and Münzer (6), Münzer and Wiener (7), Sarbo (8), Marinesco (9), Rothman (10), Rhigetti (11), Jatta (12) and others). Ballet and Dutil (13) have resorted to temporary or permanent digital compression of the abdominal aorta. Experimental embolism, by injecting lycopodium spores has been tried by Lamy (14), Singer and Rothmann (15).

[1] Received for publication October 19, 1908.

Most authors agree with Ehrlich and Brieger that one hour of ligation of the abdominal aorta produces death of the cell, whereas Spronck believes that ligation for half an hour is sufficient to produce necrosis and destruction of the cells in rabbits.

The character of the lesions is much disputed. The primary œdema described by Marinesco (9) has not been seen by other observers. Chromatolysis is admitted by most authors, the peripheral type being frequently seen. Reticular formation has been seen by many, but its exact significance is not known. As alterations of the achromatic substance the following are described: (1) Vacuolization, which is believed by Marinesco to begin at the periphery and to appear only after chromatolysis has occurred. Jatta, however, finds it to be more centrally located and already shown where the Nissl bodies are still intact. (2) Molecular disintegration and fracture of the protoplasm and dendrites are also described. (3) Most authors agree that the cell is atrophied after anæmia, although Marinesco insists on a primary œdema. (4) The nucleus is found by Sarbo to undergo acute homogeneous atrophy. Jatta found, on the other hand, karyorrhexis or disintegration of the chromatin. Ballet and Dutil, and Rothmann are the only observers who have described migration of the nucleus towards the periphery as a result either of a disaggregation of the cytoplasm or disturbance of intracellular pressure. (5) The nucleolus is generally considered to have great resistance. Swelling, atrophy, fragmentation and fusion with the nuclear substance have been described as pathological alterations.

Rhigetti is the first to call attention to the difference in the lesions occurring after a temporary or a permanent anæmia, the lesions appearing later in the latter condition, and resembling cadaveric changes.

The great resistance of the spinal ganglion cells was pointed out by Ehrlich and Brieger, and confirmed later by de Buck and de Moor. After a temporary ligation, de Buck and de Moor found around the nerve cells an infiltration of lymphoid cells which they believed to be phagocytes exuded from the lymph stream. Tuckett (16), by sectioning branches of the carotid artery and dissecting out the sheath of the sympathetic trunk, induced anæmia of the superior cervical sympathetic ganglion, the cells of which he found to possess greater resistance than those in the spinal cord. The changes within five days correspond nearly to the lesions in the cord after from twenty-four to forty-eight hours as described by Münzer and Wiener.

Mott (17) studied the changes produced in the brain of the monkey by the permanent ligation of the carotid and vertebral arteries on both sides, and found that swelling of the cell and chromolytic changes occurred within less than ten minutes after ligation. In animals that die within twenty-four hours after ligation of these arteries, the cortical ganglion cells, when stained by Nissl's method, show a uniform diffuse staining, absence of Nissl granules, an appearance of coagulating necrosis, denoting a biochemical change in the protoplasm and permanent loss of function. On the other hand, if signs of recovery are evident twenty-four hours after operation the cells are swollen, with the nucleus greatly enlarged and displaced, but there is still a differentiation into chromatic and achromatic substances, denoting biophysical rather than biochemical changes and temporary, rather than permanent, depression of function.

Later, Hill and Mott (18), using Cajal's method for neurofibrils, examined the brains of animals similarly treated, which showed signs of recovery. The neurofibrils were found to be intact, although the cells were greatly swollen.

Material and Technique.—The material was obtained, for the most part, from the animals used in the series of physiological experiments, the principal facts of which have been given in a previous paper (19), anæmia being produced by ligation of the carotid and subclavian arteries. A few cats were subjected to varying periods of anæmia, and the material taken at the desired stage of anæmia or subsequent recovery. The central nervous system was removed and pieces fixed in ninety-six per cent. alcohol and in Muller's fluid. The staining methods used were Nissl's methylene blue, with or without counterstaining with erythrosin, the Pal-Weigert and the Marchi. A two-thirds grown normal cat was killed by ether and the nervous tissue used for comparison.

The physiological deportment of the animals is given for the most part in the paper cited, the numbers of the experiments being the same in that and in the present paper. Some further facts are given in later papers (20).

The Nature of the Lesions.—The pericellular lymph space is dilated in animals in which the circulation has been allowed to return, so that it is two or three times wider than normal (Fig. 10). This extracellular œdema disappears after a certain time. After recovery is complete the lymph space is reduced to normal size. When the tissue is taken during the period of anæmia the space is rather diminished or not greater than normal in size (Fig. 9).

The contour of the cells tends to assume a somewhat rounded outline when circulation is restored. This is best seen in cells of the anterior horn of the spinal cord, whose edges are normally concave, but which become more convex from the intracellular œdema. It is hard to make out the difference between the contour of a normal cell, a cell subjected to anæmia without restoration of the circulation, and a cell which has completely recovered after being subjected to anæmia.

The subsequent increase of blood pressure when the flow returns and the probable alteration in the cement substance of the endothelium incident to the anæmia of the capillaries may possibly ex-

plain the increased transudation of lymph into the pericellular lymph spaces. The cells possibly swell by imbibition, which is favored by changes in their physical character brought about by anæmia. It has been shown by Galeotti (21) that the permeability of some cells rises sharply at death, and that other cells, *e. g.*, epithelial cells of the intestine, become permeable to ions which would not be allowed to pass under normal conditions. There is little doubt, therefore, that the permeability of the nerve cells is altered by the anæmia, and that substances in the blood and lymph which might normally be excluded now pass through the cell membrane, which has become indifferent to the ions seeking entrance or exit. There is the further possibility also that there have been formed in the nerve cells, or tissues about them, during the period of anæmia some toxic products whose presence disturbs the normal physico-chemical relationships of the cells and fluids, and which may thus give rise to the spasms seen during resuscitation. The amount of these toxic substances need not necessarily be great enough to affect distant normal nerve cells because of their presence in the blood. This idea is not incompatible with what has been observed regarding toxins in the blood of the resuscitated animals (22). In cats that die as a result of prolonged cerebral anæmia, many hours after the restoration of the blood supply, the cells are shrunken and irregular in outline, and the pericellular lymph spaces are relatively wider.

The chromatin bodies show two different kinds of lesions. One, seen in large ganglion cells (spinal cord, retina, etc.) is manifested in irregularity of outline, clumping and diffuse blue staining often extending into the nucleus, which is only marked by the presence of the relatively unchanged, deeply-stained nucleolus. As the process advances the chromatin breaks down into fine dustlike particles, which afterward become unstainable from the periphery to the center (Fig. 2). In another type of lesion, seen in the small nerve cells (small pyramidal and bipolar cells of the retina), there is a tendency to lose affinity for the stain, the granules becoming paler, and those that are stained being fewer in number.

Vacuolation of the cytoplasm occurs when the period of anæmia is long, say twenty-two minutes, and the animal survives for several hours afterwards (Fig. 6). Under these conditions the

nucleus has been seen slightly displaced towards the periphery, but we were not able to see actual extrusion of the nucleus through the cell wall.

The nucleus is often diffusely stained blue, almost uniform with the diffuse blue staining cytoplasm. In prolonged occlusion the outline becomes irregular and shrunken. A decreased affinity of the nuclear chromatin for the stain is marked in small nerve cells. We have not seen any changes in the nucleolus by the methods used.

Some nerve cells of the cat that was killed for control show chromatolytic changes which may be due to a different stage of functional activity (van Durme (23)) or to the effect of ether (Wright (24)). The average, however, is taken here for comparison.

The changes produced by occlusion of the arteries of the head vary with the length of the period of anæmia and the time that elapses after resuscitation. Chromatolysis alone, shown in tissues taken during or immediately after the experiment, does not mean death of the cell. A certain length of time must elapse after the reëstablishment of the circulation before the shrinkage of the nucleus, vacuolation and shrinkage of the protoplasm, and displacement of the nucleus, signifying solution of the achromatic substance and probable permanent impairment of the cell functions, become apparent.

The fact that a certain time must elapse after resuscitation in order to bring about the complete histological destruction of the cell is similar to the finding of Favre and Laignel-Lavastine (25), namely, that a certain interval must elapse after death before any changes in the Nissl substance of the nerve cells can be detected by histological methods.

These facts make it probable that enzymes, either within the cell or in the tissue fluids, are responsible for the final destruction of the nerve cell. This hypothesis is in harmony with the observation of Rhigetti that changes are produced sooner after temporary anæmia, possibly because of the greater amount of cytolytic enzymes that circulate when the circulation is restored; and with the findings of de Buck and de Moor of neuronophagic mononuclear leucocytes, which may furnish cytolytic enzymes when they break down.

The neurones of different animals and the neurones of the same or different regions of the same animal show different degrees of alterations with the same period of anæmia. The number of affected cells, due either to individual cellular resistance or to different collateral or lymphatic circulation, also varies.

The small pyramidal cells are the most sensitive to anæmia, showing a lessened degree of affinity to methylene blue. Eight minutes of anæmia was sufficient to kill many of the pyramidal cells of the cat of Experiment 74 (19). This animal was allowed to live three months after resuscitation. The spinal cord in the upper cervical region showed early degeneration of the pyramidal tracts. The Purkinje cells are next in the order of sensitiveness, diffuse chromatolysis being seen after thirteen minutes of anæmia.

The cells of the medulla are rather resistant. In the cat of Experiment 87, which was subjected to anæmia for twenty-two minutes, and survived twenty hours after return of circulation, the cells showed diffuse chromatolysis, with greatly diminished affinity for the stain. In this case the diffuse chromatolysis is probably in part due to profound changes in the protoplasm, by which the broken down chromatin granules, poorly stained, are diffused throughout the substance of the cell. The changes in the medulla—the seat of the respiratory and vaso-motor centers—may explain the failure to resuscitate some animals. Eight to thirteen minutes of anæmia may provoke no, or only slight, lesions. Twenty to thirty minutes may produce deeper alterations which are incompatible with complete recovery of the animal.

The retina shows about the same degree of resistance as the medulla oblongata. We have examined the retinas of cats subjected to anæmia of twelve and thirty minutes respectively, without return of the circulation, and thirteen minutes of anæmia followed by thirteen minutes of reëstablished circulation before death. The material from these three retinas does not differ to any extent. In general the ganglion cells of all of them showed a less distinct outline of the Nissl bodies, less affinity for the stain, some clumping and diffuse staining. The inner and outer granular layers were paler and of a more greenish tint when stained with methylene blue.

The cervical cord showed slight chromatolysis after an occlusion

of thirteen minutes with a subsequent reëstablishment of the circulation. More marked still were the changes in the cat of Experiment 87 (*vide supra*), the cells in the spinal cord of which showed marked chromatolysis and vacuolation. The cells of the posterior horn were less affected than those of the anterior horn. We have seen no changes in the spinal ganglia after thirty minutes of anæmia.

The neuroglia cells, although subjected to the same degree of anæmia, do not seem to be at all affected. When circulation was allowed to return, pericellular œdema, more marked around groups of cells, was seen. This is in accordance with the ideas of Cohnheim that, during anæmia of a portion of an organ, the tissues are affected in the order of the height of their differentiation.

SUMMARY AND CONCLUSIONS.

With the return of the circulation, dilation of the pericellular lymph space and slight swelling of the cell body occurs, disappearing as recovery progresses. Chromatolysis, as evidenced by poor affinity for stains, clumping, diffuse staining, and breaking into dust-like particles, induced by anæmia, is not necessarily fatal. Death of the cell is not shown histologically when tissue is removed and fixed immediately after the experiment. Some time must elapse for the detection of the vacuolation, displacement of the nucleus and solution of the chromatic substance, indicative of profound changes. Neurones from different regions as well as neurones of the same region differ in degree of resistance to anæmia. The small pyramidal cells are the most susceptible, and then come the Purkinje cells, cells of the medulla oblongata, retina, cervical cord, lumbar cord, spinal ganglia and, most resistant of all, the sympathetic ganglion cells. Failure to resuscitate animals after anæmia of the central nervous system is probably due to the destruction of many of the cells of the vital centers (vaso-motor and respiratory) which do not have histological peculiarities by which they may be defined. Death, however, of a few cells of any center does not necessarily mean the total loss of function of that center, since the remaining cells may be sufficient to discharge the function of the center.

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Explanation of Plate IV.

All the figures are drawn from cells stained by the Nissl method.

Fig. 1. Zeiss; oil imm., 2 mm.; oc. 3. A small pyramidal cell from normal cat killed by etherization.

Fig. 2. Leitz; oil imm., $\frac{1}{12}$; oc. 2. A small pyramidal cell of a cat subjected to anæmia during thirteen minutes. Tissues taken thirteen minutes after return of circulation. Showing poor affinity to stain and small number of chromatin granules.

Fig. 3. Zeiss; comp. oc. 6; oil imm.; obj. 2 mm. Large pyramidal cell from the same cat as in Fig. 2. Showing clumping or beginning chromatolysis.

Fig. 4. Zeiss; oil imm., 2 mm.; compens, oc. 6. Purkinje cell of cat subjected to twelve minutes of anæmia. Showing diffuse staining centrally and chromatolysis peripherally.

Fig. 5. Zeiss; compens, oc. 6; oil imm., 2 mm. Cell from medulla oblongata of cat used in Experiment 87; twenty-two minutes of anæmia; death within twenty hours. Showing diffuse chromatolysis and lessened affinity for stain.

Fig. 6. Leitz; obj. 7; oc. 2. Anterior horn cell of cervical spinal cord of cat of Experiment 87. Showing vacuolation and partial chromatolysis.

Plate IV which accompanies the article by Liborio Gomez and F. H. Pike on "The Histological Changes in Nerve Cells Due to Total Temporary Anæmia of the Central Nervous System" should be inserted opposite page 264.

Katherine Hill

FIG. 7. Leitz; obj. 7; oc. 2. Normal ganglion cell of retina.

FIG. 8. Zeiss; compens. oc. 6; oil imm.; obj. 2 mm. Ganglion cell from the retina of a cat subjected to thirty minutes of anæmia. Tissues taken at the end of period of occlusion. Showing clumping and beginning solution of the chromatic bodies.

FIG. 9. Zeiss; oc. 3; oil imm., 2 mm. Anterior horn cell from cervical cord of cat subjected to thirty minutes of occlusion, the circulation not being allowed to return. Shows much chromatolysis and almost complete obliteration of the pericellular lymph space.

FIG. 10. Leitz; oc. 2; oil imm., $\frac{1}{12}$. Anterior horn cell from cervical cord of cat subjected to thirteen minutes of anæmia. Killed thirteen minutes after reëstablishment of cerebral circulation. Shows chromatolysis, and intra- and extra-cellular œdema.

A STUDY OF THE PATHOLOGICAL ANATOMY OF THE PANCREAS IN NINETY CASES OF DIABETES MELLITUS.[1]

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PLATES V–VII.

Study of anatomical changes in diabetes mellitus has been limited in large part to lesions which occur in the pancreas. In 1889 were published the well-known experiments of von Mering and Minkowski (1), in which they showed that the removal of the pancreas in dogs produced typical diabetes. The diabetes of depancreatized dogs has suggested that the pancreas furnishes an internal secretion which controls carbohydrate metabolism. Since the other organs of internal secretion are ductless glands, the islands of Langerhans, the only parenchymatous tissue in the pancreas not in communication with the ducts, were associated with pancreatic internal secretion (Laguesse (2), Schäfer (3)) at a time when evidence of this association was wanting.

Opie (4), in 1900, described definite pathological changes in the islands of Langerhans in diabetes. These changes consisted most frequently in increase of fibrous tissue within and about the islands. In other cases of diabetes Opie found hyaline degeneration of the islands of Langerhans occasionally in a pancreas otherwise entirely normal. Opie reaches the conclusion that where diabetes is caused by a lesion of the pancreas, the lesion is of such a character as to injure or destroy the islands of Langerhans. These studies have been followed by a large number of articles upon the same subject, and some observers have supported, while others have opposed his views. In addition to the lesions described by Opie, other changes in the islands of Langerhans have been noted in association with

[1] Received for publication December 1, 1908.

diabetes mellitus. Schmidt (5) found acute inflammation limited to the interacinar islands in a child ten years old. Ssobolew (6) and others have reported absence of the islands of Langerhans, or great diminution in their number. Hypertrophy of these islands, associated with a peculiar adenoma-like arrangement of their cells, has been described by Reitmann (7), Herxheimer (8), MacCallum (9) and others.

Sauerbeck (10) in 1902 collected from various sources one hundred and seventy-six cases of diabetes in which the condition of the islands had been noted. He found that lesions in the islands of Langerhans had been observed in sixty-two per cent of the cases.

The present work was undertaken at the suggestion of Dr. Opie, with the hope that the study of a large number of cases by a single observer might furnish accurate information concerning the condition of the pancreas and of the islands of Langerhans in diabetes mellitus. So far as I know, only two of the following cases have been previously reported.

Gross Pathology of the Pancreas in Diabetes.—Of my cases, the pancreas has been described as small or atrophic in only twenty-five per cent. With the exception of this diminution in size, the pancreas exhibited very few notable macroscopic changes. The weight has been given in thirty-six cases, and ranges from thirty to one hundred and eighty-five grammes. The average weight was ninety-four grammes. This is scarcely less than the weight of the normal pancreas, which averages, according to Vierordt, ninety-seven grammes. The consistency of the gland in a good many cases was increased. The lobulation was indistinct in some instances and in fifteen an infiltration of fat tissue was visible with the naked eye. Neither cancer, cysts nor calculi of the pancreas were encountered. There were no cases of pancreatic apoplexy.

In considering the histological pathology of the pancreas in diabetes, it will be convenient to divide the subject into three parts: (1) Changes in the interstitial tissue. (2) Changes in the glandular acini. (3) Changes in the islands of Langerhans.

Changes in the Interstitial Tissue.—Opie (4) has shown that the interstitial changes which follow obstruction of the duct differ from

those observed in connection with diabetes. In the former, sclerosis is chiefly interlobular, and while the lobules may be invaded to a greater or less extent by the newly formed tisssue, the progress of the lesion is apparently inward from the periphery of the lobule. In the pancreatitis of diabetes, the reverse is true. The new growth originates between the acini, and interlobular sclerosis, if present at all, is a later phase of the process.

Of the ninety cases which I have studied, sixty-four, or seventy-one percent., show a definite chronic interacinar pancreatitis. In twenty-one, or twenty-four per cent., the sclerosis is marked. The distribution of this new growth is generally uneven, some lobules being more involved than others. In a number of cases this "focal" sclerosis is conspicuous. Where the induration is advanced, many of the lobules have been largely replaced by fibrous tissue, while the glandular acini which still survive are compressed and distorted, and may give evidence of atrophy. Although the newly formed fibrous tissue is often most conspicuous about the vessels and ducts, I have not been able to establish any constant relationship. In many cases, especially where the lesion is mild, the sclerosis seems more advanced in the neighborhood of the islands of Langerhans.

The relation of age to chronic interacinar inflammation is important. The lesion has been entirely absent in all of the four cases under twenty years of age. Between the ages of twenty and thirty, only twenty-five per cent. of cases show interacinar new growth. After thirty, interacinar sclerosis is the rule, being present in ninety per cent. of all cases.

Typical chronic inflammation of the interlobular variety has been observed in only four cases.

A well marked interacinar invasion was present in all four cases (Cases XXI, XXII, LII and LXII). A slight and very irregularly distributed interlobular fibrosis is seen in some severer instances of chronic intracinar inflammation, but here the process appears to be a local extension of the changes between the acini. Of the four cases just mentioned, one was associated with a duodenal ulcer, one with persistent vomiting, and another with diphtheritic colitis. In the fourth, no history was obtained. The associated intestinal lesion suggests ascending infection of the pancreatic duct.

The newly formed connective tissue which is seen in both types

of chronic interstitial pancreatitis is generally of a dense fibrous character and rather poor in spindle cells. In a number of my cases, however, the new growth is of a looser texture and quite cellular, resembling the stroma of an infant's pancreas. An extensive infiltration of lymphoid, eosinophile and plasma cells has been observed very frequently with both types of chronic interstitial pancreatitis. Of the sixty-four cases with interacinar pancreatitis, thirty have shown an abundant cellular infiltration. Lymphoid cells constitute the greater part of this infiltration, but eosinophile cells and plasma cells are also plentiful. The presence of a considerable number of cellular elements in the stroma would seem to indicate that the inflammatory process was still in the active stage. A marked infiltration of lymphoid cells was present in each of the four cases of chronic interlobular pancreatitis. Polymorphonuclear leucocytes were found in the stroma in greater or less numbers in eight cases, always associated with infiltration of lymphoid cells.

Considerable infiltration of the pancreatic stroma by adipose tissue was found in twenty-six of my cases. Three of these showed a very marked invasion, the lobules as well as many of the acini being widely separated by fatty tissue. In these three cases, the parenchyma appeared much reduced in amount and the sections gave the impression that many acini had been replaced by adipose tissue. The islands of Langerhans were very scarce in one case (Case XXXVI). In the other two (Cases L and LXXVIII) a considerable number of interacinar islands were found. One of these cases of pancreatic lipomatosis was associated with obesity.

Arteriosclerosis, it is well known, accompanies a large percentage of cases of diabetes. Of my cases, eighty per cent. showed a thickening of the wall of the pancreatic vessels and in half of these cases the vessels were also hyaline. The small arterioles seem to show this change first, and in many instances, the lumen of the smaller vessel has been almost entirely obliterated by hyperplasia of the medial coat. Hyaline changes were, moreover, more commonly observed in the small than in the large vessels.

The incidence of arteriosclerosis in diabetes increases rapidly with age. In my series, it was present in only forty per cent. of the cases between twenty and thirty years of age, increased to seventy-

seven per cent. in cases between thirty and forty, and was present in all but two cases occurring after the fortieth year.

Changes in the Secreting Parenchyma.—Clinically and experimentally, cases are constantly encountered which give no evidence of a disturbance in carbohydrate metabolism, although they show at autopsy extensive destruction of the glandular acini. According to my observations, the most common change of the acini with diabetes is the compression and atrophy, seen in association with the severer types of chronic interacinar pancreatitis. An acinus which has undergone this change is smaller than the normal; its constituent cells are pressed together and individual cells are ill defined; in some acini a number of the cells have already been destroyed, so that only a part of the original acinus remains. Many acini have been completely obliterated and their place is taken by connective tissue. In striking contrast to the atrophied acini and occasionally associated with them in the same section are acini which are larger than the normal, rich in centro-acinar cells; they suggest a compensatory hypertrophy.

The brightly staining eosinophile acini, first described by Opie, have been observed in nine of my cases. Such an acinus or group of acini, takes a bright pink stain in preparations stained with hæmatoxylin and eosin and is sharply defined from the surrounding alveoli.

Changes in the Islands of Langerhans.—In the ninety cases which I have studied, seventy-nine, or eighty-eight per cent., have shown definite anatomical changes in the islands. By far the commonest lesion is fibrosis, which has been observed in seventy-six cases. In forty it has been moderate; in thirty-six advanced. In the former cases the islands are surrounded by capsules of fibrous tissue which are thicker than the normal, almost invisible sheaths. The fibrillary coating of the insular capillaries is also definitely increased in thickness, converting the vessels into coarse septa which extend in from the capsule and anastomose at the center of the interacinar island. The cells forming the columns show little or no change in number or appearance.

The following are the cases which show *moderate* sclerosis of the islands. Cases V, X, XV, XIX, XXIV, XXV, XXXIII, XXXIV, XXXV, XXXVI,

XXXVIII, XXXIX, XL, XLI, XLII, XLIV, XLVII, XLIX, L, LI. LII, LV, LVI, LIX, LX, LXIII, LXIV, LXVIII, LXIX, LXX, LXXV, LXXVI, LXXVII, LXXVIII, LXXXII, LXXXIII, LXXXVI, LXXXVII, LXXXVIII, XC.

In the second group of cases the lesions are of a similar nature, but more advanced. The islands of Langerhans are imbedded in dense fibrous tissue which separates them widely from the surrounding alveoli. The intrainsular sclerosis still follows the path of the insular capillaries, the walls of which are several times thicker than the normal (Fig. 1). Sections through the center of the body may show an extensive patch of dense fibrous material, rich in spindle cells and sending out broad septa towards the periphery (Fig. 2). The cells of the islands are often reduced in number, and appear small and compressed. Occasionally an island of Langerhans is found which has been almost completely converted into fibrous tissue (Cases IV, IX, XXVI and LXXX).

The following are the cases which show *advanced* sclerosis of the islands. Cases I, III, IV, VI, VIII, IX, XI, XII, XIV, XVI, XVII, XVIII, XX, XXI, XXII, XXVI, XXVIII, XXIX, XXXII, XXXVII, XLIII, XLV, XLVIII, LII, LIII, LVIII, LXI, LXII, LXV, LXVII, LXXII, LXXIX, LXXX, LXXXI, LXXXIV, LXXXV.

Insular sclerosis of the mild type is usually associated with a moderate chronic interacinar pancreatitis, but this relation is not present in all cases. In four of my cases where there was no evidence of a chronic interstitial inflammation, a definite increase of connective tissue was found in the interacinar islands (Cases V, XXXV, XXXVIII and LVI). In these four cases the age varied from twenty-six to seventy years. Advanced sclerosis of the islands of Langerhans was always associated with considerable interacinar sclerosis, and the insular lesion was always most severe in those areas where the induration was most marked.

The relationship between sclerosis of the islands of Langerhans and arteriosclerosis is of interest. Fibrous changes in the islands are, with few exceptions, associated with a sclerosis of the smaller pancreatic arterioles. Of my cases of insular sclerosis, all but five showed thickening of the vessel walls; in these five cases, no arterial sclerosis was recognizable. Sclerosis of the island of Langerhans in its early stages, is probably a part of more or less generalized arteriosclerosis. It doubtless affects the capillaries of the islands of

Langerhans or their afferent vessels and in this respect resembles the beginning fibrous changes in the glomeruli of the kidney. When the process is advanced, chronic interacinar inflammation is always present and the entire interstitial framework of the island becomes involved in the new growth. The insular capillaries, however, still bear the brunt of the attack.

The average duration of diabetes mellitus in forty-six cases with sclerosis of the islands of Langerhans was three years and eleven months.

Hyaline degeneration of the islands of Langerhans is the lesion which has occurred next in frequency to sclerosis, being present in twenty-seven cases (thirty per cent.). In most instances, it has been associated with sclerosis of the pancreatic vessels and of the islands of Langerhans. There were indeed only two exceptions to this rule (Cases LXXI and LXXIV). In Case LXXI the islands were so completely destroyed that their histological structure was entirely lost (Fig. 4). In six cases with hyaline changes in the interacinar islands there was no increase in the interacinar connective tissue (Cases LXXI, XL, XLIX, LXXXVI, LIV and LXIV. As a rule the majority of the islands of Langerhans were affected, but in a few cases only an occasional island showed the change. The hyaline material is first deposited along the capillaries of the island, separating the latter from the cells. As the process advances, it extends outward. Many of the cells are replaced by the hyaline substance, and those which still survive are often compressed and atrophic. Eventually, the entire area of the island may be occupied by homogeneous hyaline material. The nuclei of the endothelial cells appear to persist longest, but finally these too are destroyed.

I have found hyaline degeneration of the islands of Langerhans in only two of the cases under thirty years of age (Cases XXXIX and LXXIV). It appears to be most common after the fifth decade. In my cases I have found that this lesion was, with the exception of case XXXIX, always associated with diabetes of more than one year's duration. The average duration of the diabetes in sixteen cases with hyaline islands was three years and six months.

In nine cases I have observed a well-marked infiltration of leucocytes about the islands of Langerhans. A more or less general

infiltration of the same character sometimes accompanied this lesion, but the cells were more numerous and concentrated in the vicinity of the interacinar islands. The cells were generally arranged in the form of a halo about the island of Langerhans just outside of the capsule, though in some cases the island itself was invaded (Fig. 3). The average duration of the diabetes in the six cases of this group where the duration was definitely stated was only eleven months.

Hypertrophy of the Islands of Langerhans.—Laguesse (2) found that the islands of Langerhans in the normal pancreas of man varied from one hundred to four hundred microns in diameter. Islands of Langerhans exceeding four hundred microns were very rare. Lydia Dewitt (11) measured these bodies in the human pancreas, and found that they averaged .0153 c.mm. in volume. In her measurements the diameter never reached 400 microns. The pancreas in thirty-eight per cent. of my cases showed islands of Langerhans which equalled or exceeded 400 microns in diameter. Round or ovoid islands were selected and the long diameter was measured.

In Case XXVI, an island of Langerhans was found which measured sixteen hundred micromillimeters—a diameter about eight times that of normal (Fig. 5). In the cases where giant islands were found, the average size of the interacinar islands was increased. Hyaline degeneration of the islands was associated with about a third of the cases with hypertrophy (see Fig. 5) and it was observed that hypertrophied islands as a rule were free from hyaline material, or contained it in only small amounts.

Reitmann (7), Herxheimer (8), MacCallum (9) and others have described a peculiar form of hypertrophy of the interacinar islands, which they have encountered in the pancreas of some cases of diabetes. The cells displayed instead of their usual arrangement in irregular cords or masses, a definite columnar arrangement (Figs. 6 and 7), and were often grouped together in circular cords about capillaries. In seven of our cases, a similar lesion has been observed. The islands of Langerhans are considerably larger than the normal, some of them reaching a diameter of seven or eight hundred microns. Their contour is quite irregular; loops and cords of columnar epithelial cells push their way out between the adjacent acini, and, but for differences in staining reaction, might possibly be mis-

taken for normal glandular structure. In one of our cases (Case XXIX) the picture is truly remarkable. The islands are very numerous, and exceedingly large and irregular in outline; their general appearance is somewhat suggestive of a neoplasm. The pancreas was normal, however, in its gross appearance and all the islands shared in the hyperplasia. In six of these cases, hypertrophy of the islands of Langerhans was associated with increase of fibrous tissue about the insular capillaries.

Number of Islands of Langerhans.—The number of islands of Langerhans in the normal pancreas varies considerably. According to Laguesse (2), the average is somewhat less than one island to each square millimeter; Sauerbeck's estimation is one island per square millimeter, and Dewitt's one and five-tenths to the square millimeter. In a number of my cases, the number of interacinar islands has been large but this apparent increase in number may have been due in many cases to increase in size of the islands, as the two phenomena were usually observed together.

Diminution in the number of islands of Langerhans was a much commoner finding. While there was no instance in my series of a pancreas entirely devoid of islands of Langerhans there were a number of specimens in which they were exceedingly few.

Several writers have found an absence or scarcity of islands in diabetes. Ssobolew (6) reported fifteen cases in which the islands were either absent altogether or were much diminished in number. Two factors probably contribute to explain this diminution. First, there is a group of cases in which the pancreas may show little or no change, and where the condition is perhaps due to a congenital defect. Opie reports the case of a child, fourteen years old, with hereditary diabetes, in which the number of islands showed a well-marked diminution. In a second group of cases, the scarcity of islands of Langerhans is associated with chronic interacinar pancreatitis of severe type. Here the islands are probably destroyed and their places occupied by fibrous tissue.

In twenty of my cases the islands of Langerhans showed a marked diminution in number, recognizable by inspection of sections. These nineteen cases may be classified as follows: (1) scarcity of islands in a normal pancreas (5 cases); (2) scarcity of islands in association with chronic interacinar pancreatitis (15 cases).

Cases in Which the Pancreas was Normal.—Eleven of my cases showed no discoverable anatomical lesion in the pancreas. The pancreas in two cases of this group (Cases VII and LVII) was described as small, but in all other respects the gross appearance agreed with the microscopic in suggesting no pathological changes. In five cases (Cases VII, XIII, LVII, XLVI and LXX) diminution in the number of interacinar islands was marked. The islands were of small size in five cases. The age in all but one of these cases was under thirty-five years.

Relationship of Diabetes to Organs other than the Pancreas.—I have already discussed the relation of pancreatic lesions occurring with diabetes to general or pancreatic arteriosclerosis.

Cirrhosis of the liver was present in seven of my cases. The pancreas from all of these cases showed well-marked chronic interacinar inflammation, which in two instances was far advanced. One etiological factor is probably active in both organs. The same statement is perhaps applicable to chronic nephritis, which was present in twenty-five cases, and was in all instances associated with more or less advanced chronic inflammation of the pancreas.

Two cases of cirrhosis of the liver were accompanied by *hæmochromatosis* (Cases XLII and LIX). This remarkable disturbance of iron metabolism was first described by von Recklinghausen (12), and is characterized by a deposition of a brown iron-containing pigment in the various tissues and organs of the body. Hæmochromatosis with diabetes or "Bronzed diabetes" is well recognized. Von Recklinghausen found cirrhosis of the liver a constant feature of the disease, and more recently a number of writers have noted the occurrence of chronic interstitial pancreatitis.

Case XLII was obtained through the kindness of Dr. MacCallum of the Johns Hopkins Hospital. A man, thirty-seven years of age, gave a history of diabetes of nine months' duration. The urine contained 6.5 per cent. of glucose. Acetone and diacetic acid were also present. There were ascites and pigmentation of the skin. The patient died in coma. At autopsy, marked pigmentation was found in the liver, heart, pancreas, lymph glands and skin. The liver showed cirrhosis and the heart was hypertrophied. The pancreas was large and firm and weighed 150 grammes. On section it was brown, and mottled with fat. Microscopically, there is a very marked infiltration of fat tissue which appears to have replaced the glandular structures in many places. This invasion is not confined to the interlobular tissue but separates the acini as well.

There is also a definite new growth of connective tissue between the acini, which is rich in spindle cells and infiltrated with a moderate number of lymphoid and plasma cells. The interstitial tissue is the seat of an abundant deposition of light brown pigment which reacts positively to the chemical test for iron. Many of the acinar cells are loaded with this pigment. Some of the acini are considerably swollen, and their cells stain faintly. The walls of the blood vessels are not appreciably thickened.

The islands of Langerhans are very scarce, and considerably smaller than the normal, none of them measuring over 200 microns in diameter. The fibrous capsules about the islands are thickened, and infiltrated by pigment. Some of the island cells also contain pigment but the remainder take their usual stain.

Case LIX was obtained through the kindness of Dr. Wright of the Massachusetts General Hospital. A man forty-five years of age had had three per cent. of glucose in his urine, as well as acetone and diacetic acid. The patient died in coma, and at autopsy there were found cirrhosis of the liver and pigmentation of the liver and pancreas. The pancreas was dark brown in color and rather soft.

Microscopically, sections from the pancreas show a marked new growth of connective tissue between the acini. The lobules are widely separated by adipose tissue. The stroma is the seat of a universal deposition of iron pigment which stains blue with ferrocyanide of potassium and hydrochloric acid. Many of the acinar cells are also loaded with pigment. The walls of the blood-vessels are somewhat thickened. Dense collections of lymphoid and polymorphonuclear cells are found in the neighborhood of the larger ducts. The islands of Langerhans are fairly numerous and normal in size; many of them are surrounded by dense capsules of fibrous tissue, and the insular capillaries are thickened. A considerable number of insular cells are distended with pigment (Fig. 8), and give evidence of degeneration, the nuclei staining indistinctly.

The islands in these two cases show very similar lesions, namely, encapsulation by fibrous tissue and a deposition of iron pigment in the insular cells (Fig. 8). In the case first described there was a marked diminution in the number of the islands of Langerhans, probably partly due to the advanced lipomatosis.

The association of diabetes with certain diseases of the ductless glands has been frequently observed. Hansemann (17) collected from the literature of the subject fifteen cases of *exophthalmic goitre* which had been accompanied by diabetes but there had been no opportunity to study the pancreas. A number of other writers have more recently observed glycosuria in connection with Grave's disease.

I have been able to include in the present series one case of diabetes which was associated with exophthalmic goitre. The case was reported by Dr. Morris Manges in the Mt. Sinai Hospital Re-

ports, Vol. II. Dr. Libman was kind enough to supply me with a part of the pancreas.

The patient, a woman, forty years of age, gave a history of diabetes of six months' duration, her chief complaint being weakness, emaciation and thirst. The urine contained five per cent. of glucose. Acidosis was present. There were marked exophthalmos, tachycardia and neuralgic pains. The latter were relieved by thyroid extract. Death occurred from asthenia and cardiac failure. At autopsy the thyroid gland was enlarged and firm; microscopically there were glandular hyperplasia and chronic inflammation. The pancreas was atrophic and weighed 45 grams; on section it was pale and soft.

Microscopically the pancreas exhibits typical chronic interacinar pancreatitis of advanced grade. The acini are small, and separated by dense fibrous tissue, which in some places has almost completely replaced the parenchyma. The stroma shows rich infiltration by lymphoid and plasma cells, with a few polymorphonuclear leucocytes. The vessel walls are thickened and hyaline. Islands of Langerhans are fairly numerous but have small area. In places where the sclerosis is severe the interacinar islands are much involved, being buried in dense fibrous tissue and invaded by the same material. Many of them have been almost obliterated by the fibrous hyperplasia.

Adenoma of the thyroid gland has been noted in the anatomical diagnosis in two of my cases with diabetes (Cases XXIX and XXXIV). It is a significant fact in this connection that in both cases the adenoma-like hypertrophy of the islands of Langerhans, described above, is present. In Cases XXIX this hypertrophy affects nearly all the islands of Langerhans, which often attain great size In Case XXXIV the insular hypertrophy is also well marked. The pancreas in both of these cases shows a well-marked chronic interacinar inflammation with considerable increase of fibrous tissue in and about the islands of Langerhans.

Myxœdema was found associated with diabetes in one of my cases (Case VI).

The patient, a woman, seventy fours years of age, was first admitted to the Presbyterian Hospital on March 12, 1899, complaining of weakness, shortness of breath, despondency, and gradual loss of mental powers.

There was a general thickening of the subcutaneous tissue. The skin was dry and scaly, and the nails and hair were brittle. There was puffiness under the eyes, and the tongue was thickened. Speech was deliberate and lisping in character. Pulse tension was high. At this time there was no glucose in the urine. The patient was treated with thyroid extract and improved rapidly.

A year and a half later, the patient was admitted again to the hospital complaining of thirst, poluria, puritus vulvæ and eczema. Myxœdema was much improved. The urine contained four per cent. of glucose. The amount of sugar diminished under treatment. During the two years following, the

patient was in the hospital several times, and finally died in coma September 6, 1902.

At autopsy the thyroid weighed only eight and a half grams; microscopically there is very advanced atrophy of the parenchyma. The alveoli have been almost completely obliterated and their places are occupied by fibrous tissue, densely infiltrated in places by lymphoid cells.

The pancreas weighed 120 grams, and was of tough consistency. Microscopically, advanced chronic interacinar inflammation, and universal infiltration of adipose tissue can be recognized in spite of extensive post-mortem digestion. The islands can be identified and show considerable sclerosis.

The occurrence of glycosuria with *acromegaly* has been observed in a considerable proportion of cases of this disease. Hansemann (17) and others have described such cases in which a chronic interstitial pancreatitis was found. Through the kindness of Dr. Norris I have been able to study the pancreas from a case of acromegaly which was accompanied by diabetes.

The case was reported by Dr. Norris in the *Proceedings* of the New York Pathological Society, February, 1907.

The patient, a man, thirty-three years old, was admitted to Bellevue Hospital with the following symptoms: Progressive enlargement of jaw, nose, ears, hands and feet. Persistent frontal headaches, and attacks of vertigo. Gradually increasing loss of vision, ending in atrophy of both optic nerves and almost complete blindness. There were recurrent epileptiform attacks. Glycosuria had been present during twenty two months.

At autopsy, a large tumor was found, connected by a broad pedicle to the pituitary gland which was itself enlarged; microscopically, the tumor is composed of cuboidal or oval cells which are arranged in irregular alveoli. The stroma is very scanty. The alveoli are separated by capillaries, whose endothelial lining is apparently in direct contact with the alveolar cells. In some places the capillaries are dilated, giving the tumor a very vascular appearance.

The pancreas was large and firm, and weighed 170 grams. The lobulation was distinct and no macroscopic lesions were noted.

Microscopically, the pancreas is free from interstitial induration. There has been some post-mortem digestion in a few places, but otherwise the glandular acini are normal in appearance. The walls of the blood-vessels are slightly thickened. The islands of Langerhans are numerous, irregular in outline, and larger than the normal; many are otherwise normal. One island is found which measures six hundred micromillimeters in diameter. Some of the larger islands present a very unusual appearance. The insular cells are large, and often columnar in shape and arrangement. In many instances the cords of cells form partial or complete circles, whose lumina contain the insular capillaries. These capillaries show definite sclerosis. There is a marked deposit of hyaline material in a few of the islands of Langerhans.

The coexistence of this adenoma-like hypertrophy of the islands

of Langerhans with adenoma of the pituitary gland is especially significant if it be borne in mind that the same type of insular hypertrophy was observed in association with the two cases of adenoma of the thyroid gland; at present I can offer no satisfactory explanation of this association.

In another case (Case LXXXIII) *calcification of the pituary gland* is mentioned as one of the anatomical findings. A well marked chronic interstitial inflammation which affects chiefly the interacinar tissue is found in the pancreas. The islands of Langerhans are small and rather scanty. They show definite fibrous changes, manifested by a thickening of the capillary walls.

Other anatomical changes associated with diabetes are of considerable interest.

Gangrene is a common complication, and is generally attributed to two causes, arterio-sclerosis and lowered resistance in the tissue, resulting from the diabetes. Gangrene occurred in sixteen of my cases and was in all instances associated with arterio-sclerosis and well marked chronic interacinar pancreatitis. The youngest individual affected by gangrene was forty-three years of age. In eleven cases it was the lower, and in two, the upper extremity which was involved. There was one case of gangrene of the scrotum and one case in which the location of the gangrene was not mentioned.

Fat necrosis has been found to occur with various lesions of the pancreas, and accompanies the acute, more often than the chronic changes. Fat necrosis is mentioned in the anatomical diagnosis of four cases in the present series. I have been unable to find any common feature other than chronic intracinar induration present in these cases.

Cholelithiasis occurred in nine of my cases. Opie (13) has shown that where gall-stones are a causal factor in chronic pancreatitis, they produce sclerosis of the interlobular variety. The inflammatory process in these nine cases was definitely interacinar; it is probable, therefore, that the gall-stones were not related etiologically to the pancreatic lesions.

PATHOGENESIS OF DIABETES MELLITUS.

The islands of Langerhans are composed of columns of cells, with a rich vascular supply, but have no communication with the ducts. In this respect they resemble the parenchyma of the thyroid and adrenal glands. If the pancreas furnishes an internal secretion, it is possible that this function is performed by the islands of Langerhans. Attempts to study these islands experimentally are met by great difficulties. Marked sclerosis of the pancreas follows ligation of the pancreatic ducts in animals but the islands of Langerhans remain normal and no glycosuria occurs. A similar lesion unaccom-

panied by diabetes occurs as the result of occlusion of the pancreatic duct in man.

A number of observers believe that the islands of Langerhans are phases in the life history of the secreting alveoli, and are not independent structures. Dale (14) claims to have converted acini into islands of Langerhans by starvation and the injection of secretin. It is possible that Dale mistook for islands groups of acini exhausted by hypersecretion and similar to areas which, as previously mentioned, occur in diabetes and fail to exhibit the staining reaction of secreting acini. The peculiar structure and blood supply of the islands of Langerhans and the characteristic staining reactions which Lane (15) has demonstrated furnish strong evidence in favor of their independence.

In the present study of the pancreas from ninety cases of diabetes, the following pancreatic changes have been encountered:

1. Chronic Inflammation of the Pancreas.	
Interacinar pancreatitis; sclerosis of islands of Langerhans	39
Interlobular pancreatitis; sclerosis of islands of Langerhans	4
Interacinar pancreatitis; hyaline degeneration of islands of Langerhans	19
Interacinar pancreatitis with lipomatosis; sclerosis of islands of Langerhans	2
Interacinar pancreatitis, with lipomatosis; hyaline degeneration of islands of Langerhans	1
Interacinar pancreatitis, with siderosis of islands of Langerhans (hæmochromatosis)	2
2. Parenchyma normal; lesions of the islands of Langerhans.	
Sclerosis of the islands of Langerhans	4
Hyaline degeneration of the islands of Langerhans	7
Infiltration of leucocytes about islands of Langerhans	1
3. Pancreas normal	11
	90

Of the eleven cases of normal pancreas, five showed a marked diminution in the number of islands of Langerhans. In two other cases the pancreas was abnormally small.

In order to establish a causal relationship between lesions of the islands of Langerhans and pancreatic diabetes, the following premises must be proven:

1. Lesions of such a nature as to interfere with the performance of their function occur in the islands of Langerhans in diabetes.

2. These lesions are primary, and not secondary to diabetes.

1. The chief object of this study has been to determine the frequency of lesions of the islands of Langerhans in diabetes. Eighty-eight per cent. of my cases have shown definite changes in the islands. In thirty per cent. of cases, some or all of the islands of Langerhans exhibited hyaline degeneration with consequent partial destruction. Lesions of the islands of Langerhans have usually been associated with chronic interacinar pancreatitis and arteriosclerosis. In twelve cases insular changes have occurred in an otherwise normal pancreas.

Are the lesions of the islands of Langerhans sufficiently severe to produce loss of function?

In most instances, there can be little doubt that hyaline degeneration and sclerosis have an injurious effect. Even where there is only a moderate thickening of the insular capillaries, the change is doubtless sufficient to interfere with the proper nutrition of the cells.

2. Are the lesions of the islands of Langerhans secondary to diabetes? There is no evidence in support of this view. The variety of lesions of these structures associated with diabetes affords evidence that they are not the result of the same cause. As a rule sclerosis of islands of Langerhans is associated with chronic interacinar pancreatitis and diabetes with such destructive lesion of the pancreas is comparable to diabetes following partial removal of the gland in lower animals.

Is the cause of pancreatic diabetes to be found in lesions of the parenchyma, in lesions of the islands of Langerhans, or in both combined? Chronic interacinar pancreatitis alone does not answer the question, for both structures are implicated. On the other hand, with forms of chronic inflammation due, for example, to duct obstruction, where the parenchyma is destroyed and the islands of Langerhans remain intact, the absence of diabetes indicates that destruction of the glandular acini is not sufficient to produce the disease.

Most significant, however, are cases with partial or complete destruction of the islands of Langerhans and no changes in the parenchyma. Twelve cases in the present series have shown lesions of this character. In seven of these the interacinar islands were hyaline, in four they were sclerotic and in one they were infiltrated by polymorphonuclear and lymphoid cells. The selective character of these lesions is strong evidence in favor of the view that the islands stand in a causal relationship to diabetes.

The frequency of hypertrophy of the islands of Langerhans in diabetes is noteworthy. In thirty-eight per cent. of my cases islands of Langerhans measuring four hundred micromillimeters or more in diameter have been found (Fig. 5). Hypertrophy of the islands

has usually been associated with sclerosis or hyaline degeneration affecting the interacinar islands in the same gland, and is probably compensatory in character.

How may the occurrence of a normal pancreas with diabetes be explained? It is possible that diabetes in some of these cases is not of pancreatic origin. It is well known that temporary glycosuria can be caused by puncture of the fourth ventricle and by other injuries of the nervous system. Falta, Eppinger and Rudinger (16) in their studies of the relationship of the pancreas to the ductless glands reach the conclusion that the latter are almost equally instrumental with the pancreas in the production of diabetes. It is significant, however, that in the cases of this series in which diabetes has accompanied lesions of the ductless glands, for example, exophthalmic goitre and myxœdema, well-marked changes in the pancreas and in the islands of Langerhans have been found.

It has been said by Sauerbeck that the occurrence of normal pancreas associated with diabetes mellitus does not furnish evidence that lesions of the islands of Langerhans do not cause glycosuria, for it is equally unfavorable to the view which refers glycosuria to changes in the secreting parenchyma. Diminution in the number and size of the islands of Langerhans in an otherwise normal pancreas may play an important part in the production of diabetes, particularly in young individuals; and a deficiency of this kind is perhaps congenital.

CONCLUSIONS.

1. Anatomical lesions of the pancreas occur in more than seven-eighths of all cases of diabetes mellitus.

2. In diabetes associated with lesions of the pancreas, the islands of Langerhans constantly show pathological changes (sclerosis, hyaline degeneration, infiltration with leucocytes and hypertrophy).

3. In some cases of pancreatic diabetes (twelve of ninety cases) the lesion of the pancreas is limited to the islands of Langerhans.

4. In sixteen cases of diabetes associated with hyaline degeneration of the islands of Langerhans the average duration of the disease has been three and a half years; in forty-six cases with sclerosis of these bodies, three years and eleven months. In six cases of diabetes associated with an infiltration of leucocytes about the

islands of Langerhans the average duration has been eleven months.

5. Destructive lesions of the islands of Langerhans may be associated with compensatory hypertrophy of other interacinar islands.

6. Peculiar adenoma-like hypertrophy of the islands of Langerhans occurs in a small proportion of cases (seven of ninety) and may be associated with adenomata of the thyroid gland (two cases) and of the pituitary body (one case).

7. Diabetes mellitus occurring in association with hæmochromatosis (bronzed diabetes) is referable to pigmentation and destruction of the islands of Langerhans.

8. The pancreas is found to exhibit no pathological changes in twelve per cent. of cases. In approximately one-half of these cases it has been noted that the size of the gland or the number of islands is much less than normal.

9. Fifty per cent. of cases of diabetes mellitus occurring before the age of thirty years are associated with lesions of the pancreas; seventy-five per cent. of all cases of diabetes in which the pancreas is normal occur before the age of thirty years. Ninety-seven per cent. of cases of diabetes occurring after the age of thirty years are associated with lesions of the pancreas; and eighty-six per cent. occur in association with chronic interacinar pancreatitis accompanying arteriosclerosis.

10. Interacinar pancreatitis which occurs in seventy-three per cent. of all cases of diabetes is almost constantly associated with arteriosclerosis; gangrene of the extremities, which occurs with one-fourth of all cases of interacinar pancreatitis, is doubtless referable to the same cause.

11. Chronic interlobular pancreatitis, when associated with diabetes, is accompanied by sclerosis or hyaline degeneration of the islands of Langerhans.

12. Diabetes in association with myxœdema or with exophthalmic goiter may be referable to a lesion of the pancreas, namely, chronic interacinar inflammation with sclerosis of the islands of Langerhans; diabetes in association with acromegaly may be referable to a lesion of the islands of Langerhans, namely, sclerosis and hyaline degeneration with adenoma-like hypertrophy.

In closing I wish to express my gratitude to Dr. Opie for much

assistance and many valuable suggestions. I am indebted to Dr. Thacher for twenty-one cases collected in this laboratory; to Dr. Wright for eighteen cases from the Massachusetts General Hospital; to Dr. Mallory for five cases from the Boston City Hospital and one case from the Long Island Hospital; to Dr. Libman for eight cases from Mt. Sinai Hospital; to Dr. Longcope for three cases from the Pennsylvania Hospital; to Dr. Oertel for six cases from the New York City Hospital; to Dr. Norris for two cases from Bellevue Hospital; to Dr. Adami and to Dr. Klatz for twelve cases from the Royal Victoria Hospital of Montreal; to Dr. Duval and Dr. White for five cases from the Montreal General Hospital; to Dr. MacCallum for five cases from the Johns Hopkins Hospital; to Dr. Wood for one case from St. Luke's Hospital; and to Dr. James and Dr. Stein for material from private cases.

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CASES OF DIABETES MELLITUS.

I. Pres. H., S. S., 72 yrs., M. Pan.—112 gms. Interacinar pancreatitis. I. of L.—Sclerotic hypertrophied. Arteries—Sclerotic, hyaline.

II. Pres. H., Mann., 10 yrs., M. Diab.—8 mos. Pan.—30 gms.; normal. I. of L.—Normal. Arteries—Normal.

III. Pres. H., L. S., 69 yrs., M. Pan.—120 gms. Interacinar pancreatitis, I. of L.—Sclerotic, hyaline, hypertrophied. Arteries—Sclerotic. Chronic nephritis.

IV. Pres. H., Dres., 71 yrs., F. Diab.—22 yrs. Pan.—100 gms. Interacinar pancreatitis. I. of L.—Very sclerotic, hypertrophied. Arteries—Sclerotic. Chronic nephritis.

V. Pres. H., Malm., 24 yrs., M. Diab.—2½ mos. Pan.—60 gms.; secreting parenchyma normal. I. of L.—Sclerotic, infiltrated with lymphoid cells, hypertrophied. Arteries—Normal.

VI. Pres. H., Pag., 74 yrs., F. Diab.—2 yrs. Pan.—120 gms. Interacinar pancreatitis. I. of L.—Very sclerotic. Arteries—Sclerotic, calcified. Myxœdema.

VII. Pres. H., Bon., 32 yrs., M. Diab.—3 yrs. Pan.—30 gms.; normal. I. of L.—Normal, very few in number. Arteries—Normal.

VIII. Pres. H., Bert., 51 yrs., M. Diab.—Several yrs. Pan.—120 gms. Interacinar pancreatitis. I. of L.—Sclerotic. Arteries—Sclerotic, hyaline. Gangrene of scrotum.

IX. Pres. H., Flie., 58 yrs., M. Marked interacinar pancreatitis. I. of L.—Sclerotic, hypertrophied. Arteries—Very sclerotic, hyaline. Cholelithiasis.

X. Pres. H., Dem., 51 yrs., M. Diab.—2 yrs. Pan.—60 gms. Interacinar pancreatitis. I. of L.—Sclerotic, infiltrated with lymphoid cells. Arteries—Sclerotic. Cholelithiasis.

XI. Pres. H., Brus., 60 yrs., F. Pan.—60 gms. Interacinar pancreatitis. I. of L.—Very sclerotic, hypertrophied. Arteries—Sclerotic, hyaline. Fat necrosis, cholelithiasis.

XII. Pres. H., Sno., 57 yrs., F. Diab.—1 yr. Pan.—90 gms. Interacinar pancreatitis. Polymorphonuclear leucocytes in stroma of pancreas. I. of L.—Sclerotic, hyaline, hypertrophied. Arteries—Sclerotic, hyaline. Chronic nephritis.

XIII. Pres. H., Sill., 27 yrs., M. Diab.—3 yrs. Pan.—60 gms.; normal. I. of L.—Small, otherwise normal; very few in number. Arteries—Normal.

XIV. Pres. H., Bot., 30 yrs., F. Pan.—45 gms. Interacinar pancreatitis. I. of L.—Very sclerotic, hyaline. Arteries—Sclerotic, hyaline.

XV. Pres. H., Lind., 35 yrs., M. Diab.—9 mos. Pan.—120 gms. Interacinar pancreatitis. Polymorphonuclear leucocytes in stroma of pancreas. I. of L.—Sclerotic, few in number. Arteries—Sclerotic.

XVI. Pres. H., Whel., 60 yrs., F. Pan.—100 gms. Interacinar pancreatitis. I. of L.—Very sclerotic, hypertrophied; adenoma-like hypertrophy. Arteries—Sclerotic. Chronic nephritis.

XVII. Pres. H., La T., 52 yrs., M. Diab.—15 mos. Pan.—185 gms. Interacinar pancreatitis. I. of L.—Very sclerotic; few in number. Arteries—Sclerotic, hyaline. Gangrene of foot.

XVIII. Pres. H., Odg., 75 yrs., F. Interacinar pancreatitis. I. of L.—Very sclerotic, hyaline. Arteries—Sclerotic. Chronic nephritis.

XIX. Pres. H., War., 27 yrs., M. Pan.—90 gms. Interacinar pancreatitis. I. of L.—Very small, sclerotic; few. Arteries—Sclerotic. Nephritis.

XX. Pres. H., Van W., 64 yrs., M. Pan.—60 gms. Interacinar pancreatitis. I. of L.—Very sclerotic; few in number. Arteries—Sclerotic, hyaline. Gangrene of foot. Chronic nephritis.

XXI. Case of Dr. James, 36 yrs., F. Diab.—About 1 yr. Interlobular pan-

creatitis. Polymorphonuclear leucocytes in stroma. I. of L.—Very sclerotic, hypertrophied. Arteries—Sclerotic. Persistent vomiting. Fat necrosis.

XXII. Pres. H., Mar. '90. Marked interlobular pancreatitis. Several miliary tubercles in stroma of pancreas. I. of L.—Very sclerotic; few in number. Arteries—sclerotic, hyaline.

XXIII. Royal V. H., 55—'97, 20 yrs., M. Diab.—"Acute course." Pan.—"Simple atrophy"; secreting parenchyma normal. I. of L.—Infiltrated with polymorphonuclear and lymphoid cells, hypertrophied. Insular cells show typical adenoma-like arrangement in a few instances. Arteries—Normal.

XXIV. Royal V. H., 73—'97, 31 yrs., M. Diab.—"Acute course." Pan.—40 gms.; "marked atrophy." Interacinar pancreatitis; infiltration of many polymorphonuclear leucocytes. I. of L.—Sclerotic, hypertrophied; cells columnar, with adenoma-like arrangement. Arteries—Sclerotic, hyaline. Fat necrosis.

XXV. Royal V. H., 32—'00, 65 yrs., M. Diab.—4 yrs. (at least). Pan.—150 gms. Interacinar pancreatitis. I. of L.—Sclerotic, hyaline, hypertrophied. Arteries—Sclerotic, hyaline. Gangrene of foot.

XXVI. Royal V. H., 95—'02, 75 yrs., M. Diab.—15 yrs. Pan.—175 gms. Interacinar pancreatitis. I. of L.—Very sclerotic, large. One very large island shows adenoma-like hypertrophy (Fig. 6). Arteries—Sclerotic, hyaline. Gangrene; cirrhosis of liver; chronic nephritis; atrophic adrenals.

XXVII. Royal V. H., 44—'03, 52 yrs., M. Diab.—15 yrs. Pan.—"Shrunken." Interacinar pancreatitis. I. of L.—Very sclerotic, hypertrophied. Arteries—Sclerotic hyaline. Chronic nephritis.

XXVIII. Royal V. H., 96—'03, 29 yrs., M. Diab.—18 mos. Pan.—150 gms. Interacinar pancreatitis. I. of L.—Very sclerotic, hypertrophied. Arteries—Sclerotic, hyaline. Cirrhosis of liver; chronic nephritis; obesity.

XXIX. Royal V. H., 79—'06, 70 yrs., M. Diab.—2 yrs. Interacinar pancreatitis. I. of L.—Very sclerotic, hypertrophied; cells columnar, with adenoma-like arrangement. Arteries—Sclerotic. Fat necrosis; adenoma of thyroid.

XXX. Royal V. H., 130—'06, 21 yrs., F. Diab.—1 yr. Pan.—Normal. I. of L.—Normal. Arteries—Normal. Chronic nephritis.

XXXI. Royal V. H., 15—'07, 15 yrs., M. Diab.—Discovered on day of death. Pan.—Normal. I. of L.—Normal. Arteries—Normal. Œdema of lungs, persistent thymus, lymphatic hyperplasia. 1.5 per cent. glucose in urine.

XXXII. Royal V. H., 102—'07, 54 yrs., F. Pan.—"Atrophied." Interacinar pancreatitis. I. of L.—Sclerotic, hyaline. Arteries—Sclerotic, hyaline. Cirrhosis of liver.

XXXIII. Royal V. H., 144—'07, 56 yrs., M. Diab.—7 yrs. Pan.—60 gms. Interacinar pancreatitis. I. of L.—Sclerotic, hyaline. Arteries—Sclerotic, hyaline.

XXXIV. Royal V. H., 14—'08, 38 yrs., F. Diab.—3 yrs. Pan.—"Atrophy." Interacinar pancreatitis. I. of L.—Sclerotic, infiltrated with lymphoid cells; adenoma-like hypertrophy. Arteries—Sclerotic. Adenoma of thyroid.

XXXV. Mont. Gen. H., '07—89, 28 yrs., M. Diab.—7 wks. Pan.—45 gms.; secreting parenchyma normal. I. of L.—Sclerotic, hypertrophied. Arteries—Some of them surrounded by leucocytes. Marked loss of weight.

XXXVI. Mont. Gen. H., '07—64, 58 yrs., M. Lipomatosis; interacinar pancreatitis. I. of L.—Sclerotic, hypertrophied; few. Arteries—Sclerotic. Chronic nephritis; obesity (great gain in weight).

XXXVII. Mont. Gen. H., '07—48, 66 yrs., F. Diab.—2½ yrs. Interacinar pancreatitis. I. of L.—Sclerotic, hyaline, hypertrophied. Arteries—Sclerotic, hyaline. Gangrene of feet; chronic nephritis.

XXXVIII. Mont. Gen. H., '08—86, 26 yrs., M. Diab.—8 wks. Pan.—"Small." No sclerosis; infiltration of lymphoid cells. I. of L.—Sclerotic, infiltrated with leucocytes, hypertrophied. Arteries—Sclerotic. Chronic nephritis.

XXXIX. Mont. Gen. H., '08—9, 26 yrs., M. Diab.—6 mos. Interacinar pancreatitis. I. of L.—Very small, sclerotic, hyaline. Arteries—Sclerotic. Chronic nephritis.

XL. Johns Hop. H., 2152, 70 yrs., M. Diab.—7 yrs. Pan.—101 gms. Normal. I. of L.—Sclerotic, hyaline. Arteries—Sclerotic, hyaline. Chronic nephritis.

XLI. Johns Hop. H., 2670. Pan.—38 gms.; normal. I. of L.—Small, otherwise normal; few in number. Arteries—Normal.

XLII. Johns Hop. H., 2782, 37 yrs., M. Diab.—9 mos. "Acute course." Pan.—150 gms. Interacinar pancreatitis; lipomatosis; siderosis. I. of L.—Small, sclerotic, infiltrated with iron pigment; few in number. Arteries—Normal. Hæmochromatosis; cirrhosis of liver.

XLIII. Johns Hop. H., 2929, 43 yrs., F. Pan.—"Areas of atrophy." Interacinar pancreatitis. I. of L.—Sclerotic, hyaline. Arteries—Sclerotic, hyaline. Gangrene of leg.

XLIV. Johns. Hop. H., 2954, 57 yrs., M. Diab.—8 yrs. Pan.—40 gms. Interacinar pancreatitis. I. of L.—Sclerotic. Arteries—Sclerotic. Cystic thyroid; gangrene of foot; chronic nephritis.

XLV. Mt. Sinai H., Hein., 58 yrs., M. Diab.—10 yrs. Interacinar pancreatitis. I. of L.—Very sclerotic. Arteries—Sclerotic, hyaline. Chronic nephritis.

XLVI. Mt. Sinai H., Isr., 44 yrs., F. Pan.—185 gms.; normal. I. of L.—Normal; very few in number. Arteries—Normal.

XLVII. Mt. Sinai H., Kop., 32 yrs., F. Interacinar pancreatitis; polymorphonuclear leucocytes in stroma. I. of L.—Sclerotic. Arteries—Sclerotic, hyaline.

XLVIII. Mt. Sinai H., Hirs., 44 yrs., F. Diab.—6 mos. Pan.—45 gms. Interacinar pancreatitis. I. of L.—Very sclerotic. Arteries—Sclerotic, hyaline. Exophthalmic goitre.

XLIX. Mt. Sinai H., Kauf., 56 yrs., M. Pan.—"Small"; secreting parenchyma normal. I. of L.—Sclerotic, hyaline. Arteries—Sclerotic, hyaline.

L. Mt. Sinai H., Ster., 75 yrs., M. Diab.—10 yrs., at least. Pan.—100 gms. Interacinar pancreatitis; lipomatosis. I. of L.—Sclerotic, hypertrophied. Arteries—Sclerotic, hyaline. Death from hemorrhage into gastro-intestinal tract. (No break in mucosa found.)

LI. Mt. Sinai H., Fat., 50 yrs., M. Diab.—Several years. Interacinar pancreatitis. I. of L.—Sclerotic. Arteries—Sclerotic, hyaline.

LII. Mass. Gen. H., 823, 45 yrs., F. Diab.—6 mos. Pan.—"Much diminished in size." Marked interlobular pancreatitis. I. of L.—Very sclerotic; few in number. Arteries—Sclerotic. Diphtheritic colitis; chronic nephritis.

LIII. Mass. Gen. H., 831, 58 yrs., F. Diab.—2 yrs. Interacinar pancreatitis. I. of L.—Very sclerotic, hyaline. Arteries—Sclerotic, hyaline. Cholelithiasis.

LIV. Mass. Gen. H., 833, 66 yrs., M. Diab.—9 yrs. Pan.—No sclerosis; rich infiltration of lymphoid cells. I. of L.—Hyaline, hypertrophied. Arteries—Sclerotic.

LV. Mass. Gen. H., 839, 53 yrs., F. Diab.—9 mos. Interacinar pancreatitis (slight). I. of L.—Sclerotic. Arteries—Sclerotic.

LVI. Mass. Gen. H., 1050, 70 yrs., F. Pan.—Secreting parenchyma normal. I. of L.—Sclerotic. Arteries—Sclerotic, hyaline. Senile kidney and liver.

LVII. Mass. Gen. H., 1094, 20 yrs., M. Diab.—3 wks. Pan.—"Small"; normal. I. of L.—Normal, but small, and few in number. Arteries—Normal.

LVIII. Mass. Gen. H., 1202, 60 yrs., M. Diab.—4 wks. Pan.—"Rather small." Marked interacinar pancreatitis; polymorphonuclear leucocytes in stroma of pancreas. I. of L.—Very sclerotic; few in number. Arteries—Sclerotic, hyaline. Gangrene of foot.

LIX. Mass. Gen. H., 1358, 45 yrs., M. Interacinar pancreatitis; siderosis. I. of L.—Sclerotic, infiltrated with iron pigment. Arteries—Sclerotic. Hæmochromatosis; cirrhosis of liver.

LX. Mass. Gen. H., 1493, 48 yrs., F. Pan.—"Rather small." Interacinar pancreatitis. I. of L.—Sclerotic, hyaline. Arteries—Sclerotic. Gangrene of arm, syphilitic (?) hepatitis.

LXI. Mass. Gen. H., 1595, 56 yrs., F. Diab.—15 yrs. Interacinar pancreatitis. I. of L.—Very sclerotic. Arteries—Sclerotic, hyaline.

LXII. Mass. Gen. H., 1643, 59 yrs., M. Pan.—"Rather small." Interlobular pancreatitis. I. of L.—Sclerotic, infiltrated with lymphoid cells; some larger than usual. Arteries—Sclerotic. Duodenal ulcer.

LXIII. Mass. Gen. H., 1770, 39 yrs., F. Diab.—Several yrs. Interacinar pancreatitis. I. of L.—Sclerotic, small. Very few in number. Arteries—Sclerotic. Gangrene of foot.

LXIV. Mass. Gen. H., 1855, 60 yrs., M. Diab.—1 yr. (at least). Pan.—Secreting parenchyma normal. I. of L.—Sclerotic, hyaline. Arteries—Sclerotic. Chronic cholecystitis.

LXV. Mass. Gen. H., 1890, 60 yrs., F. Diab.—Symptoms for 4 or 5 mos. Pan.—"Quite small." Interacinar pancreatitis. I. of L.—Sclerotic, infiltrated with lymphoid cells. Arteries—Sclerotic. Cholelithiasis.

LXVI. Mass. Gen. H., 1891, 30 yrs., M. Diab.—2 yrs. Interacinar pancreatitis; suppurative inflammation about ducts of pancreas. I. of L.—Sclerotic, infiltrated with lymphoid cells; few in number. Arteries—Normal.

LXVII. Mass. Gen. H., 1909, 68 yrs., F. Diab.—5–6 yrs. Interacinar pancreatitis. I. of L.—Sclerotic, hyaline, hypertrophied. Arteries—Sclerotic, hyaline. Gangrene of foot, fat necrosis.

LXVIII. Mass. Gen. H., 1917, 52 yrs., M. Diab.—1 yr. Interacinar pancreatitis. I. of L.—Sclerotic, hyaline, hypertrophied. Arteries—Sclerotic. Gangrene of toe.

LXIX. Mass. Gen. H., 2047, 31 yrs., M. Diab.—5 yrs. Interacinar pancreatitis. I. of L.—Sclerotic; very few in number. Arteries—Sclerotic.

LXX. Bost. City H., '08—73, 27 yrs., M. Normal. I. of L.—Normal; small and very few in number. Arteries—Sclerotic. Very severe diabetes.

LXXI. Bost. City H., '02—164, 43 yrs., M. Diab.—2 yrs. Pan.—Secreting parenchyma normal. I. of L.—Hyaline, hypertrophied. Arteries—Normal.

LXXII. Bost. City H., '07—82, 59 yrs., F. Diab.—6 yrs. Interacinar pancreatitis. I. of L.—Very sclerotic, hyaline, hypertrophied. Arteries—Sclerotic. Cholelithiasis; chronic cholecystitis.

LXXIII. Bost. City H., '99—20, 9 yrs., F. Diab.—8 mos. Pan.—Normal. I. of L.—Normal, and large. Arteries—Normal.

LXXIV. Long Isl. H., 4186, 12 yrs., M. Diab.—1 yr. Pan.—Secreting parenchyma normal. I. of L.—Many hyaline. Arteries—Normal.

LXXV. Penn. H., 785, 57 yrs., F. Diab.—2 yrs. Interacinar pancreatitis. I. of L.—Sclerotic, hypertrophied; few. Arteries—Sclerotic, hyaline. Goiter.

LXXVI. Penn. H., 938, 70 yrs., F. Diab.—4 yrs. Pan.—90 gms. Interacinar pancreatitis. I. of L.—Sclerotic, hyaline, hypertrophied. Arteries—Sclerotic. Cholelithiasis; chronic cholecystitis; chronic nephritis.

LXXVII. Penn. H., 608, 36 yrs., F. Pan.—90 gms. Interacinar pancreatitis. I. of L.—Sclerotic, hypertrophied. Arteries—Sclerotic. Cholelithiasis.

LXXVIII. N. Y. City H., Doy., 50 yrs., F. Diab.—9 mos. in hospital. Pan.—"Small." Interacinar pancreatitis; lipomatosis. I. of L.—Sclerotic, hyaline, hypertrophied. Arteries—Sclerotic, hyaline. Gangrene of foot; chronic nephritis.

LXXIX. N. Y. City H., Schro., 69 yrs., M. Interacinar pancreatitis. I. of L.—Sclerotic. Arteries—Sclerotic, hyaline. Chronic nephritis.

LXXX. N. Y. City H., Nst., 65 yrs., F. Pan.—100 gms. Interacinar pancreatitis. I. of L.—Small, sclerotic; very few in number. Arteries—Sclerotic. Cirrhosis of liver; chronic nephritis.

LXXXI. N. Y. City H., Fsh., 56 yrs., M. Marked interacinar pancreatitis. I. of L.—Very sclerotic. Arteries—Sclerotic. Atrophy of adrenals; gangrene of leg.

LXXXII. N. Y. City H., Mar., 47 yrs., M. Diab.—1 yr. Interacinar pancreatitis. I. of L.—Sclerotic. Arteries—Sclerotic, hyaline. Chronic nephritis.

LXXXIII. N. Y. City H., Con., 60 yrs., M. Diab.—3¾ yrs. Pan.—"Small." Interacinar pancreatitis. I. of L.—Small, sclerotic; very few in number. Arteries—Sclerotic, hyaline.

LXXXIV. N. Y. City H., Hen., 51 yrs., F. Interacinar pancreatitis. I. of L.—Very sclerotic, hyaline. Arteries—Sclerotic. Gangrene of hand; obesity.

LXXXV. Case of Dr. Stein's, 43, yrs., M. Diab.—4 yrs. Interacinar pancreatitis; in places, stroma of pancreas shows dense infiltration of polymorphonuclear leucocytes. I. of L.—Very sclerotic. Arteries—Sclerotic.

LXXXVI. Bellevue H., 1163, 33 yrs., M. Diab.—22 mos. Pan.—170 gms. Normal. I. of L.—Sclerotic, hyaline, hypertrophied; some show adenoma-like hypertrophy. Arteries—Sclerotic. Acromegaly; adenoma of pituitary body.

LXXXVII. Bellevue H., 1100, 73 yrs., M. Diab.—6 mos. Interacinar pancreatitis. I. of L.—Very sclerotic, hyaline. Arteries—Sclerotic, hyaline.

LXXXVIII. Mt. Sinai H., Nov., '08, 73 yrs., M. Pan.—180 gms. Interacinar pancreatitis. I. of L.—Sclerotic, hyaline. Arteries—Sclerotic, hyaline.

LXXXIX. Bost. City H., '05—172, 21 yrs., M. Pan.—Normal. I. of L.—Normal. Arteries—Normal.

XC. St. Luke's H. Interacinar pancreatitis. Stroma of pancreas densely infiltrated with polynuclear leucocytes. I. of L.—Sclerotic, infiltrated with leucocytes. Arteries—Normal.

EXPLANATION OF PLATES V–VII.

These photographs have been made by Dr. Edward Leaming at the Rockefeller Institute for Medical Research.

FIG. 1. Sclerosis of island of Langerhans; newly formed fibrous tissue about capillaries (Case XXXVIII).

FIG. 2. Sclerosis of island of Langerhans; central mass of new fibrous tissue (Case LXI).

FIG. 3. Island of Langerhans surrounded and infiltrated with lymphoid cells (Case XXXIV).

FIG. 4. Hyaline degeneration of islands of Langerhans; secreting parenchyma normal (Case LXXI).

FIG. 5. Hyaline degeneration together with simple hypertrophy of islands of Langerhans (Case XXXVII).

FIG. 6. Enormous adenomatous hypertrophy of an island of Langerhans (Case XXVI); the diameter is 1600 microns, magnification being the same as that of Figs. 4 and 5. The enlarged island is well preserved, though the surrounding tissue exhibits self-digestion.

FIG. 7. Adenoma-like hypertrophy of island of Langerhans (Case XXIX).

FIG. 8. Pigmentation of island of Langerhans with hæmochromatosis (Case LIX).

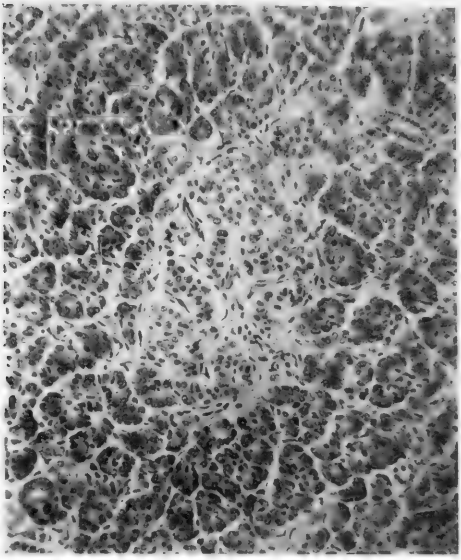

FIG. 1.

FIG. 2.

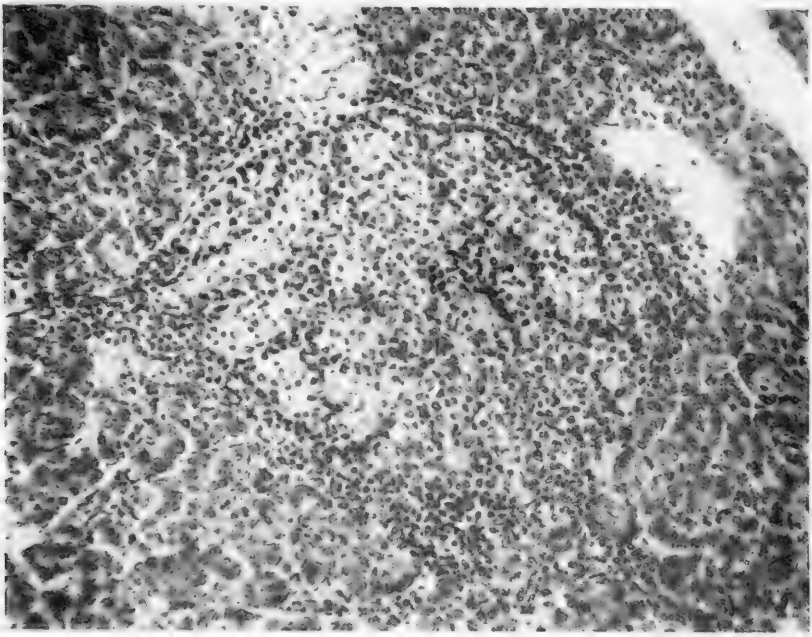

FIG. 3.

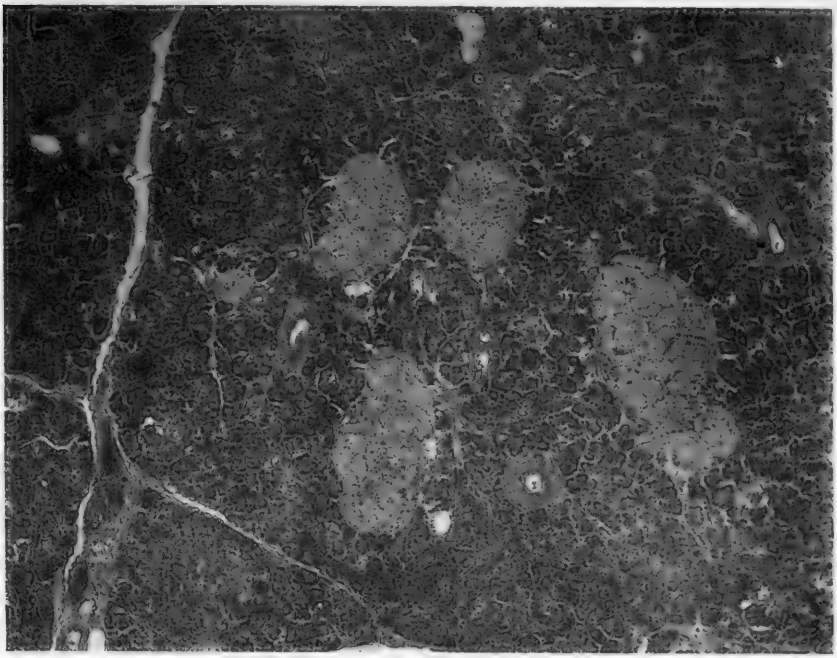

FIG. 4.

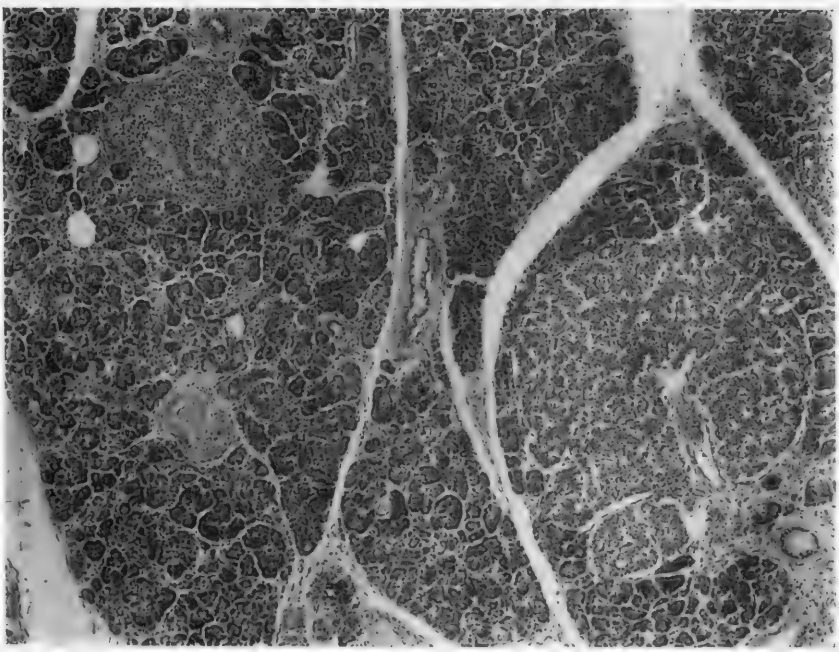

FIG. 5.

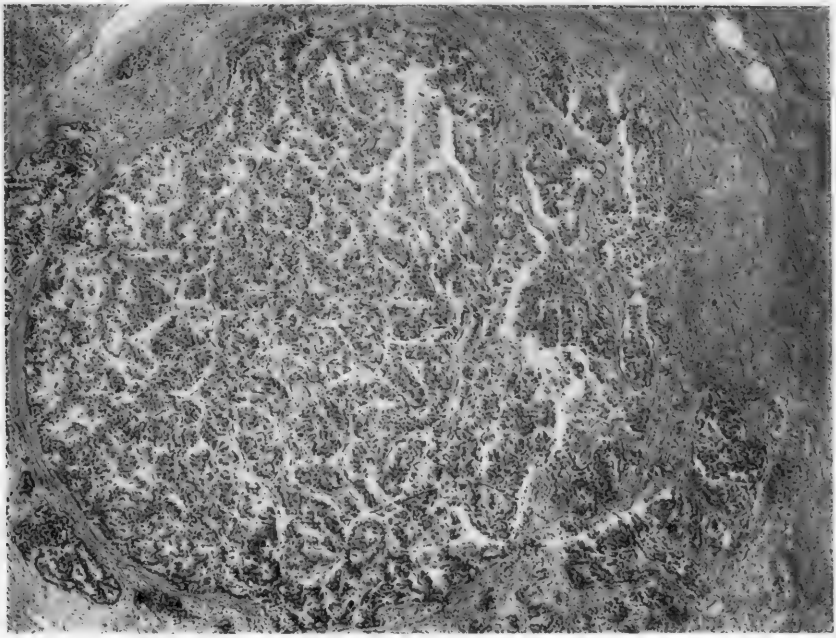

FIG. 6.

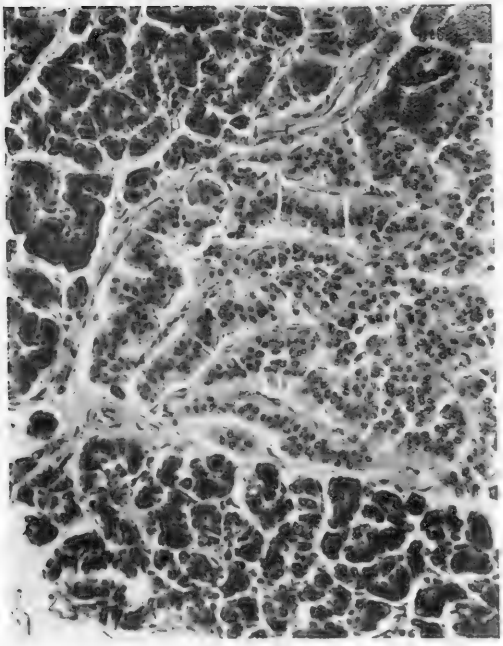

FIG. 7.

FIG. 8.

STUDIES IN EDEMA.

I. Comparative Investigation into the Action of Calcium Chloride and Sodium Chloride on the Production of Urine, Intestinal Fluid and Ascites.[1]

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Continuing the work of Jacques Loeb (1) on the antagonistic action of certain salt solutions on cellular functions, John Bruce MacCallum (2) in the laboratory of J. Loeb found that an intravenous injection of pure calcium chloride solution inhibits diuresis caused by the intravenous injection of solutions of sodium chloride and certain other salts. He (3) also has found that calcium chloride has an inhibitory effect upon the secretion[2] of fluid into the intestinal canal whether inside the body or *in vitro*. Whether the mode of action of calcium chloride consists in its direct influence upon the permeability of cells, especially of certain gland cells, as MacCallum believed, or whether its influence is merely an indirect one, excited through change in the circulatory system, we shall not here discuss.

The following experiments were undertaken in order to determine whether or not calcium chloride has the same inhibitory action upon the production of edema, especially of ascites, in experimentally produced hydræmic plethora. Does calcium chloride influence the endothelial cells of the blood vessels or of the peritoneal cavity in a way that would lead to an alteration of the permeability of the endothelial surfaces or to an alteration of a real secretory function of the cells, if such secretory function should exist, in a similar

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[2] The word secretion is used here and at some other places throughout this paper not as implying necessarily a specific cellular function, but as indicating in a general way the transmission of fluid through an epithelial surface.

manner as it influences directly or indirectly the renal cells and intestinal mucosa? Or if calcium chloride acts mainly through the circulatory system do the changes in the circulation caused by its injection affect alike the secretion of urine and intestinal fluid and the production of ascites?

To determine this question we injected into one set of rabbits considerable quantities of pure sodium chloride solutions and into another set mixtures of isotonic sodium chloride and calcium chloride solutions. This plan of injecting the calcium chloride solutions seems more perfectly comparable to natural conditions than an alternating injection of the two solutions, since under natural conditions calcium chloride is always associated with sodium chloride in the living organism.

In all the experiments rabbits were used, and with but few exceptions, the weight of these ranged between 1,600 and 1,800 grams, although it was found in the course of the experiments that the weight of the animal had no influence on the amount of ascites, intestinal fluid or urine. Most of the animals had been made to fast for twenty-four hours previous to the experiment, but nevertheless a small amount of solid matter was always found in the small intestines; in a few unfasting animals, which were killed for other purposes, the intestinal contents which were mostly solid were rarely found to exceed ten cubic centimeters.

In all cases where the intestinal contents were measured, the intestines were closed, before the infusion was started, near the pylorus and at the ileo-cecal valve by vein clamps, they having first been emptied by light stripping of as much of their contents as was possible without inflicting injury to the mucosa.

In those cases where it was desired that the kidneys should not functionate, the renal vessels and ureter were ligatured, the operation being done, after anesthesia, through an anterior incision into the peritoneal cavity. Although at first the kidneys were removed, this was later given up on account of the impossibility of preventing some of the blood from the operation getting into the peritoneal cavity.

Blood counts were taken during the course of the experiments. In the earlier experiments these were made from the blood of the

ear vein, but we found this unreliable on account of the congestion and stasis which resulted from the continued dependent position of the ears. We therefore decided to use the jugular vein for the blood determinations. At first both hæmoglobin and red corpuscle counts were made—the first by the Fleischl-Miescher hæmoglobinometer, the latter by the usual Thoma apparatus. We came to rely in our later experiments on the hæmoglobin estimations alone. The first counts were always taken before the animal was anesthetized in order to avoid the increase of erythrocytes which results from the etherization (4, 5, 6). The second count was taken during the course, or at the end of the experiment, and the percentage of decrease of hæmoglobin or erythrocytes was determined.

The fluid was infused through a canula in the right jugular vein. The fluid was allowed to flow in from a 100 cubic centimeter burette which was kept in an oblique position and which was filled when necessary from a larger reservoir; from this burette the fluid passed through a metal coil which was in a hot water bath; further on there was a thermometer bathed by the solution indicating the temperature which was kept between 35° and 39° C. The rate of inflow was controlled by a screw clamp and this rate was always, except in a few cases when otherwise stated, approximately four cubic centimeters per minute.

The fluids used were a solution of 0.85 per cent. sodium chloride and solutions of 1.11 per cent., 1.22 per cent.,[3] and 1.45 per cent. calcium chloride. The calcium chloride solution was used only in combination with sodium chloride solution, usually in proportion of one volume of the former to five volumes of the latter. The quantities infused varied between 500 c.c. and 1,000 c.c., but in the majority of the experiments 600 c.c. or 800 c.c. were allowed to flow in.

When it was desired to measure the quantity of urine in male rabbits a catheter was passed into the bladder; in female animals the urine was expressed from the bladder every few minutes by gentle pressure. Since we were measuring the urine for periods of twenty-five minutes and we usually obtained large quantities of urine, these methods were considered sufficiently exact.

[3] An 1.22 per cent. $CaCl_2$ solution ($CaCl_2$ free from water of crystallization being used) is approximately isotonic with an 0.85 per cent. NaCl solution.

The ascites was removed at the end of the experiment by opening widely the abdomen. The error of the measurement did not exceed five cubic centimeters. To obtain the intestinal fluid the clamped small intestines were removed from the body and the intestines lightly stripped. The contents were allowed to settle in a graduate and after approximately twenty hours the relation between solid and fluid contents was determined. The former usually represented only a small fraction of the fluid. The solid matter was subtracted from the total intestinal contents, and the remainder was called the intestinal fluid and used exclusively for computing the results.

INFUSIONS INTO NON-NEPHRECTOMIZED ANIMALS.

In the first series the intestinal fluid was not determined; in the second series the intestinal fluid was measured. In the first series of non-nephrectomized animals infused with pure NaCl solution we find per 1,000 c.c. of retained fluid, 88 c.c. of ascites and 670 c.c. of urine (we obtained the figure for the "fluid retained" by subtracting the urine from the injected fluid). If we exclude one exceptional case in which there were 280 c.c. of ascites (an amount never approached in any other case) the ascites is reduced to 35 c.c. per 1,000 c.c. of retained fluid; in the exceptional case the amount of urine excreted was very small and there is reason to assume that some pathological conditions were present in this animal.

In the second series we find per 1,000 c.c. of retained fluid 810 c.c. of urine, 46 c.c. of ascites and 53 c.c. of intestinal fluid.

Considering both series (when the averages of several series or groups are to be obtained the results of all experiments in both or all the series or groups are added and the figures per 1,000 c.c. made up from these sums) we obtain for each 1,000 c.c. of fluid retained 69 c.c. of ascites and 730 c.c. of urine (the intestinal fluid is noted only in the second series) and for every 1,000 c.c. of inflow of NaCl solution 420 c.c. of urine.

In the first series of $CaCl_2$ experiments in which $CaCl_2$ solutions of 1.11 per cent. were used in proportions of 1 volume of $CaCl_2$ solution to 7, 5 or 3 volumes of NaCl solutions, the urine per 1,000 c.c. of retained fluid is 207 c.c. and the ascites, 94 c.c.

TABLE I.

Non-Nephrectomized Animals Infused with 0.85 per cent. NaCl Solution.

First Series.

Inflow.	Ascites.	Urine.
768 c.c.	0 c.c.	520 c.c.
787 c.c.	8 c.c.	464 c.c.
480 c.c.	50 c.c.	110 c.c.
1022 c.c.	0 c.c.	723 c.c.
440 c.c.	20 c.c.	0 c.c.
982 c.c.	0 c.c.	490 c.c
1024 c.c.	15 c.c.	365 c.c.
792 c.c.	12 c.c.	128 c.c.
912 c.c.	280 c.c.	135 c.c.

Per 1,000 c.c. retained: Ascites 88 c.c.
Per 1,000 c.c. retained: Urine 670 c.c.

Second Series.

Inflow.	Ascites.	Int. Fld.	Urine.
900 c.c.	10 c.c.	12 c.c.	690 c.c.
900 c.c.	2 c.c.	8 c.c.	550 c.c.
800 c.c.	10 c.c.	5 c.c.	441 c.c.
640 c.c.	18 c.c.	56 c.c.	250 c.c.
800 c.c.	15 c.c.	8 c.c.	366 c.c.
900 c.c.	15 c.c.	35 c.c.	411 c.c
820 c.c.	40 c.c.	20 c.c.	320 c.c.
800 c.c.	54 c.c.	25 c.c.	176 c.c.
720 c.c.	23 c.c.	45 c.c.	68 c.c.

Per 1,000 c.c. retained fluid: Ascites 46 c.c.
Per 1,000 c.c. retained fluid: Int. Fld. 53 c.c.
Per 1,000 c.c. retained fluid: Urine 810 c.c.

First and Second Series.

Per 1,000 c.c. retained fluid: Ascites 68 c.c.
Per 1,000 c.c. retained fluid: Urine 730 c.c.
Per 1,000 c.c. infused fluid: Urine 420 c.c.

In the second series in one group in which $CaCl_2$ solution of 1.11 per cent. was used in mixtures of 1 volume of $CaCl_2$ solution to 4 volumes of NaCl solution, the urine was 240 c.c., the ascites 101 c.c. and the intestinal fluid 56 c.c. per 1,000 c.c. retained. In another group where a $CaCl_2$ solution of 1.45 per cent in a mixture of 1 volume of $CaCl_2$ solution and 5 volumes of NaCl solution was used we find per 1,000 c.c. of retained fluid, 180 c.c. of urine, 86 c.c. of ascites and 28 c.c. of intestinal fluid. In a third group where a $CaCl_2$

solution of 1.22 per cent. in a mixture of 4 volumes of NaCl solution was used, the urine was 430 c.c., the ascites 123 c.c. and the intestinal fluid 25 c.c. per 1,000 c.c. of retained fluid.

Summarizing the experiments with non-nephrectomized animals treated with NaCl—$CaCl_2$ mixtures we find per 1,000 c.c. of fluid retained, 240 c.c. of urine, 98 c.c. of ascites and (in the second series) 44 c.c. of intestinal fluid. The urine per 1,000 c.c. of infused fluid is 190 c.c.

We find therefore a marked decrease of the urine when $CaCl_2$ solutions were added to the NaCl solutions, also a decrease in the amount of intestinal fluid, but on the other hand an increase of ascites when the NaCl—$CaCl_2$ solutions were infused. However, the decrease in the amount of urine is considerably more marked than is the decrease in the amount of intestinal fluid.

TABLE II.

Using 7 Vol. 0.85 per cent. NaCl and 1 Vol. 1.11 per cent. $CaCl_2$.

Inflow.	Ascites.	Urine.
158 c.c.	0 c.c.	3 c.c.
443 c.c.	8 c.c.	20 c.c.
500 c.c.	90 c.c.	65 c.c.
500 c.c.	55 c.c.	0 c.c.
811 c.c.	90 c.c.	300 c.c.
700 c.c.	50 c.c.	112 c.c.
1062 c.c.	130 c.c.	235 c.c.
864 c.c.	70 c.c.	13 c.c.

Using 5 Vol. 0.85 per cent. NaCl and 1 Vol. 1.11 per cent. $CaCl_2$.

100 c.c.	0 c.c.	0 c.c.
190 c.c.	0 c.c.	0 c.c.
466 c.c.	40 c.c.	77 c.c.
500 c.c.	0 c.c.	144 c.c.
600 c.c.	115 c.c.	125 c.c.
1000 c.c.	10 c.c.	500 c.c.
654 c.c.	46 c.c.	358 c.c.
800 c.c.	60 c.c.	193 c.c.
700 c.c.	225 c.c.	80 c.c.
900 c.c.	110 c.c.	37 c.c.

Using 3 Vol. 0.85 per cent. NaCl and 1.11 per cent. $CaCl_2$.

Inflow.	Ascites.	Urine.
180 c.c.	0 c.c.	72 c.c.
214 c.c.	13 c.c.	30 c.c.
232 c.c.	0 c.c.	10 c.c.
277 c.c.	0 c.c.	30 c.c.
331 c.c.	25 c.c.	0 c.c.
338 c.c.	10 c.c.	54 c.c.
462 c.c.	20 c.c.	80 c.c.
500 c.c.	2 c.c.	70 c.c.
614 c.c.	35 c.c.	180 c.c.
450 c.c.	30 c.c.	0 c.c.
1039 c.c.	40 c.c.	380 c.c.
1080 c.c.	100 c.c.	55 c.c.

Per 1,000 c.c. fluid retained: Ascites 94 c.c.
Per 1,000 c.c. fluid retained: Urine 207 c.c.

Second Series.

Using 4 Vol. 0.85 per cent. NaCl and 1 Vol. 1.11 per cent. $CaCl_2$.

Inflow.	Ascites.	Int. Fld.	Urine.
350 c.c.	10 c.c.	25 c.c.	13 c.c.
800 c.c.	20 c.c.	8 c.c.	393 c.c.
850 c.c.	15 c.c.	3 c.c.	417 c.c.
650 c.c.	85 c.c.	20 c.c.	24 c.c.
800 c.c.	75 c.c.	14 c.c.	140 c.c.
800 c.c.	85 c.c.	98 c.c.	106 c.c.
800 c.c.	90 c.c.	35 c.c.	42 c.c.
800 c.c.	95 c.c.	62 c.c.	26 c.c.

Per 1,000 c.c. fluid retained: Ascites 101 c.c.
Per 1,000 c.c. fluid retained: Int. Fld. 56 c.c.
Per 1,000 c.c. fluid retained: Urine 240 c.c.

Using 5 Vol. 0.85 per cent. NaCl and 1 Vol. 1.45 per cent. $CaCl_2$.

450 c.c.	35 c.c.	21 c.c.	10 c.c.
600 c.c.	55 c.c.	7 c.c.	50 c.c.
800 c.c.	45 c.c.	16 c.c.	230 c.c.

Per 1,000 c.c. fluid retained: Ascites 86 c.c.
Per 1,000 c.c. fluid retained: Int. Fld. 28 c.c.
Per 1,000 c.c. fluid retained: Urine 180 c.c.

Using 4 Vol. 0.85 per cent. NaCl and 1 Vol. 1.22 per cent. $CaCl_2$.

500 c.c.	52 c.c.	10 c.c.	150 c.c.
620 c.c.	42 c.c.	8 c.c.	245 c.c.
780 c.c.	78 c.c.	10 c.c.	255 c.c.
360 c.c.	38 c.c.	10 c.c.	130 c.c.
500 c.c.	45 c.c.	15 c.c.	105 c.c.

Per 1,000 c.c. fluid retained: Ascites 123 c.c.
Per 1,000 c.c. fluid retained: Int. Fld. 25 c.c.
Per 1,000 c.c. fluid retained: Urine 430 c.c.

First and Second Series.

Per 1,000 c.c. fluid retained:	Ascites	98 c.c.
Per 1,000 c.c. fluid retained:	Int. Fld.	44 c.c. (2nd series)
Per 1,000 c.c. fluid retained:	Urine	240 c.c.
Per 1,000 c.c. fluid infused:	Urine	190 c.c.

In order to make the experiments still more comparable we carried on a series of experiments with both NaCl and NaCl—$CaCl_2$ solutions in which the same quantities of fluid were retained in the two series and compared the amounts of ascitic and intestinal fluid in these experiments. Comparing the results of these two groups of five experiments we find per 1,000 c.c. of fluid retained 41 c.c. of ascitic and 47 c.c. of intestinal fluid when the animals were infused with pure NaCl solutions and 123 c.c. of ascitic and 25 c.c. intestinal fluid when they were infused with NaCl—$CaCl_2$ solutions; certainly a marked difference between the two solutions which, however, merely confirms our former results.

TABLE III.

Comparison of Ascites and Intestinal Fluid in Animals Infused with NaCl Solution or Mixtures of NaCl—$CaCl_2$ with like Amounts of Fluid Retained.

	NaCl.		$CaCl_2$.	
Fluid Retained.	Ascites.	Int. Fld.	Ascites.	Int. Fld.
350 c.c.	2 c.c.	8 c.c.	52 c.c.	10 c.c.
390 c.c.	10 c.c.	5 c.c.	42 c.c.	8 c.c.
390 c.c.	18 c.c.	56 c.c.	45 c.c.	15 c.c.
430 c.c.	15 c.c.	8 c.c.	38 c.c.	10 c.c.
500 c.c.	40 c.c.	20 c.c.	78 c.c.	10 c.c.
Per 1,000 c.c. retained fluid:	41 c.c.	47 c.c.	123 c.c.	25 c.c.

But even in these experiments we have one variable factor which differs in the experiments with NaCl and those with NaCl—$CaCl_2$, namely, the time necessary for the same amount of fluid to be retained. The time necessary for the same amount of fluid to be retained is greater in the NaCl series, for here more urine is secreted; this is equivalent to a slower rate of inflow in the NaCl series. As we shall see later on, the rate is of some importance in determining the amount of intestinal fluid but the rate of inflow influences the amount of ascitic fluid only to a relatively slight de-

gree. This time factor would be unable to account for the great differences in ascitic fluid which we find here between the series with NaCl and $CaCl_2$ solutions. This conclusion is strengthened through the fact that $CaCl_2$, as will be shown in the following experiment, exerts the same effect in nephrectomized animals in which the time factor is eliminated.

INFUSIONS INTO NEPHRECTOMIZED ANIMALS.

The first series in which 0.85 per cent. NaCl solution was used includes experiments in which various amounts of fluid from 500 c.c. to 1,000 c.c. were infused. We note a progressive increase of the ascites with the increase in the amount of fluid infused; namely, between 500 c.c. and 600 c.c. of infused fluid, 50 c.c. of ascites; between 600 and 700 c.c., 62 c.c. of ascites; between 700 and 800 c.c., 75 c.c. of ascites; and between 800 and 900 c.c., 95 c.c. of ascites; but the ascites increases in proportion more than does the amount of infused fluid. The intestinal fluid also increases with the amount of infused fluid, but more irregularly. Between 500 c.c. and 600 c.c. of infused fluid, we find 50 c.c. of intestinal fluid; between 600 and 700 c.c., 60 c.c. of intestinal fluid; between 700 and 800 c.c., 72 c.c. of intestinal fluid; and between 800 and 900 c.c., 85 c.c. of intestinal fluid. The intestinal fluid seems to increase in proportion less than the amount of fluid injected and certainly does not increase as rapidly as does the ascites.

Considering only the experiments in this first series, with an inflow of either 600 or 800 c.c., we find at 600 c.c. of inflow 106 c.c. of ascitic fluid and 81 c.c. of intestinal fluid per 1,000 c.c. of infused fluid; and at 800 c.c., 91 c.c. of ascites and 99 c.c. of intestinal fluid per 1,000 c.c. of infused fluid.

For all the experiments in this first set of infusions of NaCl solution into nephrectomized animals we find per 1,000 c.c. of infused fluid 109 c.c. of ascitic and 102 c.c. of intestinal fluid.

Considering now the second series in which experiments with only 600 c.c. or 800 c.c. of inflow were made, we find for 600 c.c. of infused solution 109 c.c. of ascites and 76 c.c. of intestinal fluid and for 800 c.c. of infused solution 147 c.c. of ascites and 76 c.c. of intestinal fluid, per 1,000 c.c. of fluid injected.

TABLE IV.

Nephrectomized Animals Infused with NaCl Solution.

First Series.

Inflow.	Ascites.	Int. Fld.
500 c.c.	25 c.c.	30 c.c.
	50 c.c.	40 c.c.
	62 c.c.	70 c.c.
	Average, 45.6 c.c.	46.6 c.c.
600 c.c.	46 c.c.	40 c.c.
	85 c.c.	57 c.c.
	60 c.c.	50 c.c.
	Average, 63.6 c.c.	49 c.c.
700 c.c.	95 c.c.	85 c.c.
	80 c.c.	90 c.c.
730 c.c.	70 c.c.	60 c.c.
750 c.c.	63 c.c.	60 c.c.
750 c.c.	53 c.c.	110 c.c.
750 c.c.	55 c.c.	55 c.c.
780 c.c.	104 c.c	13 c.c.
800 c.c.	60 c.c.	63 c.c.
	98 c.c.	80 c.c.
	85 c.c.	53 c.c.
	49 c.c.	65 c.c.
	69 c.c.	75 c.c.
	65 c.c.	90 c.c.
	75 c.c.	43 c.c.
	55 c.c.	64 c.c.
	90 c.c.	83 c.c.
	Average, 72.6 c.c.	79 c.c.
900 c.c.	150 c.c.	75 c.c.
	125 c.c.	85 c.c.
940 c.c.	60 c.c.	60 c.c.
945 c.c.	150 c.c.	143 c.c.
950 c.c.	80 c.c.	140 c.c.
1000 c.c.	100 c.c.	100 c.c.

Per 1,000 c.c. infused fluid: Ascites 109 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 102 c.c.

Second Series.

Inflow.	Ascites.	Int. Fld.
600 c.c.	71 c.c.	68 c.c.
	64 c.c.	47 c.c.
	31 c.c.	55 c.c.
	74 c.c.	57 c.c.
	77 c.c.	35 c.c.
	60 c.c.	35 c.c.
	80 c.c.	34 c.c.
	Average, 65.3 c.c.	47.3 c.c.
800 c.c.	50 c.c.	82 c.c.
	75 c.c.	75 c.c.
	88 c.c.	74 c.c.
	155 c.c.	62 c.c.
	150 c.c.	58 c.c.
	135 c.c.	110 c.c.
	138 c.c.	30 c.c.
	136 c.c.	12 c.c.
	110 c.c.	88 c.c.
	147 c.c.	60 c.c.
	98 c.c.	38 c.c.
	135 c.c.	110 c.c.
	117 c.c.	70 c.c.
	Average, 118 c.c.	66.7 c.c.

Per 1,000 c.c. infused fluid: Ascites 131 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 82 c.c.

First and second series
Per 1,000 c.c. infused fluid: Ascites 109 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 94 c.c.

Considering those experiments in which 600 c.c. or 800 c.c. were infused into the animal in both the first and second series, we find the average per 1,000 c.c. of injected fluid at 600 c.c. of inflow 114 c.c. of ascites and 79 c.c. of intestinal fluid and at 800 c.c. of inflow, 124 c.c. of ascites and 90 c.c. of intestinal fluid.

Adding all experiments with various amounts of inflow we find per 1,000 c.c. of injected fluid 109 c.c. of ascitic fluid and 94 c.c. of intestinal fluid.

It may be noted that in the second series of experiments we find the figures for the amount of ascites considerably higher than in the earlier series, this being most marked with an inflow of 800 c.c.

The second series was done during the early summer, in June, when the average temperature was high, while the first series was done during the winter months. It is possible that differences in season are of importance. Comparable to our observation is perhaps the fact noted by Meltzer and Salant (7) that absorption from the peritoneal cavity is slower during the summer months than during the cooler seasons.

In the first set of experiments with the injection of mixtures of NaCl and $CaCl_2$ solutions into nephrectomized animals some were done with mixtures of 1 volume of 1.11 per cent. $CaCl_2$ and 4 or 5 volumes of 0.85 per cent. NaCl, some with mixtures of 1 volume of 1.45 per cent. $CaCl_2$ solution and 5 volumes of NaCl solution. In these experiments in which the less concentrated $CaCl_2$ solutions were used, we find 118 c.c. of ascites and 76 c.c. of intestinal fluid per 1,000 c.c. of infused fluid; in those in which the stronger $CaCl_2$ solutions were employed, 130 c.c. of ascitic and 60.3 c.c. of intestinal fluid per 1,000 c.c. infused. Thus it would seem that the stronger $CaCl_2$ solution increased the ascites and lessened the intestinal fluid more markedly than did the weak solution.

Considering those experiments in which 600 c.c. of the solution were allowed to flow in, we find 133 c.c. of ascites and 62 c.c. of intestinal fluid per 1,000 c.c. of inflow; with 800 c.c. of inflow, 150 c.c. of ascites and 58 c.c. of intestinal fluid, also per 1,000 inflow.

In the second series, where the infused fluid was a mixture of 1 volume of 1.22 per cent. $CaCl_2$ solution and 4 volumes of NaCl solution, we find per 1,000 c.c. of infused fluid 185 c.c. of ascites and 76 c.c. of intestinal fluid, corresponding to 600 c.c. of inflow and 208 c.c. of ascites and 27 c.c. of intestinal fluid to 800 c.c. of inflow.

Considering the experiments in both series in which there was 600 c.c. or 800 c.c. of inflow, we find per 1,000 c.c. retained 170 c.c. of ascites and 69 c.c. of intestinal fluid at 600 c.c. of inflow, and 180 c.c. of ascites and 47 c.c. of intestinal fluid at 800 c.c. of infused fluid.

In these experiments with NaCl—$CaCl_2$ mixtures, we note as in the NaCl experiments that the amounts of ascitic fluid in the second series are greater than in the first series and the same explanation

perhaps holds in this case, namely, the influence of the season during which the experiments were performed.

Comparing now those experiments on nephrectomized animals into which NaCl solutions were infused with those on nephrectomized rabbits into which $CaCl_2$ mixtures were infused, we note that in those infused with NaCl solution the ascites and intestinal fluid are approximately the same or the intestinal fluid is but slightly less; in the experiments with NaCl—$CaCl_2$ mixtures, the ascites is considerably greater than the intestinal fluid, and the amount of ascitic fluid is greater and the amount of intestinal fluid smaller in the $CaCl_2$—NaCl series than in the NaCl series. The $CaCl_2$ has caused a decrease in the amount of the intestinal and an increase in the amount of the ascitic fluid.

Referring to the observations above in regard to the amount of intestinal fluid after 600 c.c. or 800 c.c. have been allowed to flow in, we note that in the animals infused with NaCl solutions we obtain 79 c.c. intestinal fluid at 600 c.c. and 90 c.c. intestinal fluid at 800 c.c., while in the animals infused with $CaCl_2$ solution we obtain 69 c.c. intestinal fluid at 600 c.c. and 47 c.c. intestinal fluid at 800 c.c. In the animals injected with NaCl solutions, the intestinal fluid has increased between 600 c.c. and 800 c.c. of inflow, and in the animals injected with NaCl—$CaCl_2$ solutions, it has decreased between 600 c.c. and 800 c.c. inflow. We must consider the amount of fluid in the intestinal cavity dependent upon two factors —the secretion into and the absorption from the intestinal cavity; the amount found at the end of the experiment represents the difference between those two factors. Our experiments do not permit any analysis of those two factors. The fact that we find less intestinal fluid after 800 c.c. inflow than after 600 c.c. of inflow of NaCl—$CaCl_2$ solution is accounted for by assuming that in using mixtures of NaCl—$CaCl_2$, the absorption from the intestinal cavity is less inhibited than is the secretion into this cavity.

Both Höber (8) and Cushny and Wallace (9) have shown that the fluid from solutions of $CaCl_2$ is absorbed more slowly by the intestines than is the fluid of NaCl solutions. It may therefore be that the secretion into the intestine becomes gradually more inhibited under the influence of $CaCl_2$ than the absorption from the

TABLE V.

Nephrectomized Animals Infused with NaCl—$CaCl_2$ Solution.

First Series.

Using 4 Vols. of 0.85 per cent. NaCl and 1 Vol. 1.11 per cent. $CaCl_2$.

Inflow.	Ascites.	Int. Fld.
450 c.c.	45 c.c.	22 c.c.
530 c.c.	67 c.c.	15 c.c.
600 c.c.	63 c.c.	29 c.c.
700 c.c.	65 c.c.	52 c.c.
800 c.c.	92 c.c.	84 c.c.
"	103 c.c.	75 c.c.

Using 5 Vols. of 0.85 per cent. NaCl and 1 Vol. 1.11 per cent. $CaCl_2$.

500 c.c.	95 c.c.	—
600 c.c.	92 c.c.	37 c.c.
770 c.c.	75 c.c.	72 c.c.
800 c.c.	110 c.c.	—
"	85 c.c.	—
"	117 c.c.	68 c.c.

Per 1,000 c.c. infused fluid: Ascites 118 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 76 c.c.

Using 5 Vols. of 0.85 per cent. NaCl and 1 Vol. 1.45 per cent. $CaCl_2$.

	350 c.c.	40 c.c.	10 c.c.
	380 c.c.	45 c.c.	33 c.c.
	470 c.c.	72 c.c.	20 c.c.
	550 c.c.	50 c.c.	40 c.c.
	600 c.c.	70 c.c.	30 c.c.
	600 c.c.	85 c.c.	43 c.c.
	600 c.c.	90 c.c.	47 c.c.
Average,	600 c.c.	81.6 c.c.	40 c.c.
	700 c.c.	45 c.c.	61 c.c.
	740 c.c.	80 c.c.	70 c.c.
	750 c.c.	99 c.c.	14 c.c.
	770 c.c.	105 c.c.	70 c.c.
	800 c.c.	145 c.c.	35 c.c.
	800 c.c.	95 c.c.	33 c.c.
	800 c.c.	103 c.c.	55 c.c.
	800 c.c.	117 c.c.	16 c.c.
	800 c.c.	180 c.c.	57 c.c.
Average,	800 c.c.	128 c.c.	39.2 c.c.

Per 1,000 c.c. infused fluid: Ascites 130 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 60.3 c.c.

Experiments with both 1.11 per cent. and 1.45 per cent. $CaCl_2$ solutions.

Per 1,000 c.c. infused fluid: Ascites 127 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 65 c.c.

Second Series.

Using 4 Vols. NaCl and 1 Vol. 1.22 per cent. $CaCl_2$.

Inflow.	Ascites.	Int. Fld.
600 c.c.	130 c.c.	30 c.c.
600 c.c.	115 c.c.	65 c.c.
600 c.c.	105 c.c.	60 c.c.
600 c.c.	80 c.c.	35 c.c.
600 c.c.	110 c.c.	35 c.c.
600 c.c.	125 c.c.	43 c.c.
	Average, 111 c.c.	44.6 c.c.
800 c.c.	155 c.c.	21 c.c.
800 c.c.	162 c.c.	22 c.c.
800 c.c.	210 c.c.	36 c.c.
800 c.c.	150 c.c.	20 c.c.
800 c.c.	180 c.c.	15 c.c.
800 c.c.	140 c.c.	20 c.c.
800 c.c.	170 c.c.	53 c.c.
	Average, 166.7 c.c.	27 c.c.

Per 1,000 c.c. infused fluid: Ascites 193 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 49 c.c.

First and second series

Per 1,000 c.c. infused fluid: Ascites 145 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 59 c.c.

intestines. Whether $CaCl_2$ has the same inhibitory effect upon the absorption from the peritoneal cavity that it has upon absorption from the intestines has to our knowledge not been investigated. If $CaCl_2$ should decrease the absorption from the peritoneum, the increase in ascites which we find in our experiments under the influence of $CaCl_2$ might, in part at least, be accounted for by the decrease in the absorption from the peritoneal cavity and may not be entirely due to increased transudation into the peritoneum.

Returning now to the experiments with non-nephrectomized animals, we note in these experiments the same general facts, namely, that the ascites is greater with $CaCl_2$ solution, the intestinal fluid is greater with NaCl solution, and the relation of ascitic fluid to the

intestinal fluid is greater under the influence of $CaCl_2$. The $CaCl_2$ acts more strongly in inhibiting the action of the kidneys than it does in the intestines for it reduced the amount of urine secreted by more than half, it reduces the amount of intestinal fluid only by a quarter to a third.

If we compare the experiments on the nephrectomized and non-nephrectomized animals, we find in the former per 1,000 c.c. of retained fluid (which is equal to the infused fluid) 109 c.c. of ascitic and 94 c.c. of intestinal fluid with NaCl solution and 145 c.c. of ascitic and 59 c.c. of intestinal fluid with NaCl—$CaCl_2$ mixtures. In the latter we find per 1,000 c.c. retained fluid 68 c.c. of ascites and 53 c.c. of intestinal fluid with NaCl solution and 98 c.c. of ascitic and 44 c.c. of intestinal fluid with NaCl—$CaCl_2$; thus there are considerably less ascites and intestinal fluid in the non-nephrectomized than in the nephrectomized animals per 1,000 c.c. retained. It is to be noted that in the experiments with non-nephrectomized animals the ascitic and intestinal fluids have been considered in relation to the amount of fluid retained, not the amount of fluid infused; thus the fluid lost by secretion of urine has been taken into consideration, and the conditions in both sets of experiments are comparable. In connection with this series the observations of Magnus (10) may be of interest. This investigator found that nephrectomy performed several hours or days previous to the infusion of fluids led to edema of the skin in a similar way as would the injection of certain toxic substances. He refers this effect in both cases to some injury to the blood vessels. In the case of nephrectomized animals the substances not excreted by the kidneys are believed by him to act in a similar way on the blood vessels, as *e. g.*, arsenic; since in our experiments the infusion took place immediately after the nephrectomy it is questionable whether there was sufficient time for injury to the vessels to be thus produced. Meltzer and Auer (11) offer as an explanation of the increased absorption from the peritoneal cavity following nephrectomy an increased osmotic pressure of the blood due to retained urinary salts; but such an increased absorption would lead to exactly the opposite result from that which we obtained in our experiments, so we cannot explain this difference between the animals with and

without functionating kidneys as due to the increase of the osmotic pressure of the blood. Changes in permeability of the blood vessels on the other hand might lead to such a result.

J. B. MacCallum, in the course of his experiments, was led to the conclusion that the fluid secreted by the intestines varied roughly with the rapidity of the injection; the difference in the amounts of intestinal fluid as obtained in nephrectomized and non-nephrectomized animals he would thus explain on the basis of the rapidity of inflow. If this be true, can the relation of time to the amount of infused fluid also explain the lessened amount of ascites in these non-nephrectomized animals? Since secretions of various quantities of urine during the course of the infusion would, in certain respects, act in a similar manner as a slower injection of fluid, we can in certain respects equalize the conditions in nephrectomized and non-nephrectomized animals by using in the case of the nephrectomized animal a slower rate of inflow, so that in a given time the same amount of fluid has been retained in nephrectomized and non-nephrectomized animals. To determine what bearing the rapidity of inflow might have, we conducted a series of experiments on nephrectomized animals in which the time consumed for a certain inflow corresponded to the time consumed in non-nephrectomized animals for the same amount of fluid to be retained. In this series we obtained in using solutions of NaCl, 81 c.c. of ascites and 48 c.c. of intestinal fluid per 1,000 c.c. infused, as compared with 109 c.c. of ascitic and 94 c.c. of intestinal fluid in nephrectomized animals at a rate of inflow of 4 c.c., and 68 c.c. of ascitic and 53 c.c. of intestinal fluid per 1,000 c.c. retained in experiments with non-nephrectomized animals, and using $CaCl_2$ mixtures of 138 c.c. of ascitic and 32 c.c. of intestinal fluid per 1,000 c.c. infused, as compared with 145 c.c. of ascitic and 59 c.c. of intestinal fluid in nephrectomized rabbits at a rate of 4 c.c. per minute and 98 c.c. of ascitic and 44 c.c. of intestinal fluid in experiments with non-nephrectomized animals. Thus with slow inflow in both the NaCl and NaCl—$CaCl_2$ series we obtained a larger amount of ascites than in the non-nephrectmized series with the usual rate of inflow, if equal amounts of fluid have been retained in the same period of time in both cases; therefore, so we may set aside the suggestion that

the decrease of transudation into the peritoneal cavity was due entirely to the decrease in the rapidity of the injection. The rapidity of inflow may nevertheless have some influence because the ascites is not so great with slow inflow as with the usual rate of inflow in nephrectomized animals. We must, however, remember that in almost all of the experiments with the slow rate of inflow only relatively small quantities of fluid were infused and we have already noted that the ascitic fluid increases disproportionately to the amount of fluid infused so that this fact may account for the smaller ascites in this group with slow inflow. We cannot, however, exclude the influence of the rate of inflow as one factor which determines the increase of ascitic fluid in nephrectomized animals.

TABLE V.

Infusions into Nephrectomized Animals at a Slow Rate of Inflow.

Infused with 0.85 per cent. NaCl Solution.

Inflow.	Rate.	Ascites.	Int. Fld.
320 c.c.	2–2½ c.c. per min.	8 c.c.	9 c.c.
430 c.c.	2 c.c. per min.	40 c.c.	18 c.c.
489 c.c.	2–2½ c.c. per min.	11 c.c.	8 c.c.
489 c.c.	2½ c.c. per min.	25 c.c.	30 c.c.
400 c.c.	2½ c.c. per min.	25 c.c.	27 c.c.
400 c.c.	2¼ c.c. per min.	48 c.c.	18 c.c.
500 c.c.	2½ c.c. per min.	71 c.c.	21 c.c.
624 c.c.	3 c.c. per min.	71 c.c.	48 c.c.

Per 1,000 c.c. infused fluid: Ascites 81 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 48 c.c.

TABLE VI.

Infused with 4 Vol. of 0.85 per cent. NaCl and 1 Vol. of 1.22 per cent. $CaCl_2$.

340 c.c.	2½ c.c. per min.	43 c.c.	4 c.c.
400 c.c.	2½ c.c. per min.	68 c.c.	14 c.c.
400 c.c.	2½ c.c. per min.	73 c.c.	20 c.c.
425 c.c.	2½ c.c. per min.	30 c.c.	18 c.c.
500 c.c.	2½ c.c. per min.	85 c.c.	19 c.c.
500 c.c.	2½ c.c. per min.	57 c.c.	8 c.c.

Per 1,000 c.c. infused fluid: Ascites 138 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 32 c.c.

TABLE VII.

Blood Counts. Taken after 600 c.c. of inflow.

Per Cent. Hæmoglobin Reduction.	Per Cent. Erythrocyte Reduction.
20 per cent.	17 per cent.
39 per cent.	28 per cent.
35 per cent.	26 per cent.
25 per cent.	28 per cent.
40 per cent.	25 per cent.
29 per cent.	24 per cent.
25 per cent.	20 per cent.
26 per cent.	
28 per cent.	
34 per cent.	
Average 30 per cent.	23 per cent.

Taken after 700 c.c. of inflow.

18 per cent.	18 per cent.
39 per cent.	35 per cent.
20 per cent.	32 per cent.
25 per cent.	18 per cent.
33 per cent.	
29 per cent.	
27 per cent.	
30 per cent.	
27 per cent.	
Average 29 per cent.	25 per cent.

Taken after 800 c.c. of inflow.

27 per cent.	19 per cent.
44 per cent.	30 per cent.
31 per cent.	13 per cent.
37 per cent.	32 per cent.
38 per cent.	28 per cent.
32 per cent.	27 per cent.
43 per cent.	26 per cent.
19 per cent.	
25 per cent.	
23 per cent.	
25 per cent.	
31 per cent.	
Average 32 per cent.	24 per cent.

We find, however, in this series with a slower rate of inflow than the usual 4 c.c. per minute that the quantity intestinal fluid is lower than in non-nephrectomized animals in both NaCl and $CaCl_2$ series

for the same amount of fluid retained. Thus the increase in intestinal fluid in nephrectomized animals may be accounted for by the relatively larger amount of fluid retained in a given time.

The effect of the infusions in diluting the blood was found to have no relation to the chemical character of the infused fluid, nor the condition of the rabbit—whether nephrectomized or not if we compare the dilution of the blood at the beginning and at the end of the experiment. The blood was taken in the manner above described in various experiments after either 600 c.c., 700 c.c. or 800 c.c. of fluid had been infused. It was found that the amount of inflow had, within the limits indicated above, but little influence upon the reduction of either hæmoglobin or erythrocytes, for, with the above amounts of inflow, the average reduction of hæmoglobin varied between 29 and 32 per cent., and of erythrocytes between 23 and 25 per cent. However, we found quite marked individual variations. In some experiments not noted here the reduction of hæmoglobin was as marked after 300 c.c. of inflow as in the above experiments after 600 to 800 c.c. of inflow.

Magnus (12) in infusing fluid into dogs found more reduction of the hæmoglobin than do we—from 25 to 66 per cent.—but since he used NaCl solutions of varying concentration, hypotonic and hypertonic as well as isotonic, the two sets of experiments are not quite analogous. It is certain that the infusions dilute the blood, but in no manner definitely referable to the chemical character of the solutions, the amount of the infused fluid or the individual animals. Yet when we consider that in these cases the amount of fluid infused is equal to as much as 6 to 7 times the amount of blood of the animal, the dilution of the blood seems insignificant and the tissues must rapidly care for a large part of the infused fluid.

In a considerable number of cases the animals died during the experiments of edema of the lung, but this occurrence was more frequent in those cases in which $CaCl_2$ solutions had been added than when pure NaCl solutions were used. Edema of the lung appeared in 25 per cent. of the NaCl experiments and in 44 per cent. of the $CaCl_2$ experiments; it was severe in 13 per cent. of the experiments with NaCl solutions and in 25 per cent. of those with NaCl—$CaCl_2$ solutions. The number of cases of edema has diminished as the

technique of the experiments has become more perfect (that is, the control of the temperature and rate of inflow) but the edema of the lung still continued to be more frequent in the experiments with $CaCl_2$ solutions.

Hydrothorax was found in a large majority of the animals. The amount of fluid, however, was usually small and its occurrence and amount had no relation to the various solutions infused. It is peculiar that Cohnheim and Lichtheim (13), although they speak of the occurrence of ascites and of edema of the organs which we also found, say they did not find hydrothorax.

Turning now to the original question whether $CaCl_2$ exerts the same influence on the endothelial cells of the peritoneal cavity as it does on the kidneys and intestines, we find this answered in the negative, for although there is an inhibition of the secretion of fluid through renal cells and into the intestinal canal we find an increase in the production of ascites. Calcium chloride may be considered to increase the ascitic fluid in two ways: (1) indirectly through diminution of the amount of urine and intestinal fluid; (2) through increasing the ascitic fluid independently of its action on the kidney, etc., inasmuch as we found a marked increase of ascites after nephrectomy. It may be noted in all the experiments with non-nephrectomized animals that there exists a certain relation between the amount of urine excreted and the amount of ascites; to a certain degree the urine and ascites increase inversely. This is true not only in the animals infused with NaCl—$CaCl_2$ mixtures but also in animals infused with pure NaCl solutions; this decrease of urine under the influence of $CaCl_2$ is, as stated above, one of the causes of the increase of ascites under the influence of $CaCl_2$. We find in nephrectomized animals that the decrease of intestinal fluid was as marked as the increase of ascites, while in the non-nephrectomized animals it was not so marked.

Attention should be called to the considerable individual variations of the animals in regard to power to bear the infusions of the fluids as well as to the production of urine, intestinal fluid and ascites; which variations necessitated the large number of experiments. However, in averaging series of nine or ten experiments

these variations were found to be practically eliminated and the average of the different experiments agree well with each other.

CONCLUSIONS.

1. The secretion of urine and the elimination of fluid through the intestinal canal which are caused by the intravenous injection of solution of 0.85 per cent. sodium chloride are decreased by the addition of calcium chloride to the sodium chloride solution. The secretion of urine is more markedly inhibited than is the elimination of fluid through the intestines.

2. In contradistinction to the decreased elimination of fluid through the kidneys and intestines, addition of calcium chloride to the sodium chloride solution increases markedly the transudation of fluid into the peritoneal cavity. To a certain degree the urine and ascites may be said to increase in an inverse proportion.

3. Although calcium chloride inhibits both absorption from and secretion into the intestines it seems to decrease the secretion more markedly than the absorption.

4. The action of calcium chloride in increasing the ascitic fluid is a double one: first, by diminishing the amount of urine secreted; secondly, by increasing the ascites independently of its action on the kidneys. The latter may be a direct action on the endothelial cells of the peritoneal cavity; this, however, must be determined by further investigations.

5. Addition of calcium chloride to the infused fluids increases the tendency to the occurrence of edema of the lungs.

6. Infusion of large quantities of fluid into animals dilutes the blood, but this dilution seems to be carried only to a certain degree—about 30 per cent.—and to be independent of the chemical character of the solution and of the function of the kidneys.

7. The presence or absence of the kidneys has a marked influence on the intestinal and ascitic fluids. When the averages of ascitic and intestinal fluids per 1,000 c.c. of retained fluid in non-nephrectomized animals and these fluids per 1,000 c.c. of infused fluid in nephrectomized animals are compared, more fluid is found in the case of the nephrectomized animals. This fact can only be explained in part by the shorter time necessary for the same amount of fluid

to be retained in the case of the nephrectomized animals. Nephrectomy causes an increase of the ascites and the intestinal fluids through a mechanism which will have to be investigated by means of further experiments.

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THE POSTMORTEM RIGOR OF THE MAMMALIAN HEART AND THE INFLUENCE OF AN ANTE-MORTEM STIMULATION OF THE PNEUMOGASTRIC NERVES UPON ITS DEVELOPMENT.[18]

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Part II.

The Effect of Stimulation of the Pneumogastric Nerves Upon Cardiac Rigor.

Introduction.—As previously stated,[19] the main object of the present research is a study of the effects of vagus stimulation upon the course, onset, and growth of cardiac rigor. The following considerations led up to the investigation of that problem. By numerous experiments and observations the following facts have been established regarding the influence of motor activity and innervation upon the appearance of rigor in skeletal muscles. Increased muscular activity hastens development of rigor. On the other hand, injury to or destruction of the central nervous system, section of motor nerves, or poisoning by curare retard the onset of rigor in the corresponding skeletal muscles. Furthermore, repeated stimulations of the peripheral end of a motor nerve hasten the development of rigor in the muscles presided over by it. This may be due simply to the muscular contraction which the stimulation produces. But according to Meirowsky[20] the onset of rigor is hastened also by an electrical stimulation of a strength insufficient to produce muscular contractions. At any rate, these facts demonstrate that *motor* impulses sent to muscles *hasten* the onset of rigor in them. The

[18] Received for publication September 18, 1908.

[19] Joseph and Meltzer, *Jour. of Exper. Med.*, 1909, i, 10.

[20] Meirowsky, *Pflüger's Archiv*, 1896, lxxviii, 64.

question which presented itself to us was: would *inhibitory* impulses, if sent ante-mortem to muscles, *retard* in them the development of rigor? For instance, if isolated nerve fibers going to skeletal muscles should be discovered, would stimulation of these inhibitory fibers retard the development of rigor in these muscles, just as stimulation of the motor nerve fibers hastens it? While with regard to skeletal muscles such a question has not yet even an academic standing, it appeared to be a very practicable problem, when the rigor of the heart muscle was taken into consideration, since the pneumogastric nerves contain inhibitory fibers the stimulation of which causes the heart to stand still in diastole.

The question which we undertook to study first was would ante-mortem stimulations of the vagus nerves, sufficiently strong to produce diastolic standstills, cause a retardation in the onset of cardiac rigor?

As far as we know, the literature on cardiac rigor contains no statement bearing directly on this question, and the problem seems to be entirely new. We may state, however, that for the question as it was formulated above, we expected to obtain an affirmative answer. For, a stimulation of the vagi which is strong enough to cause prolonged and frequent standstills of the heart, should, even without the element of inhibition, be the means of retarding the onset of rigor in the heart, simply on account of the diminished activity of that organ, since motor activity is an undoubted factor in the development of rigor in skeletal muscles.

Method.—The essential features of the method employed in this study were described in the foregoing part,[21] when reporting upon the observations made on cardiac rigor in the control (normal) animals. We have to add, however, a few data describing some special features of the pneumogastric experiments.

After etherizing the animal, both vagi and both carotid arteries were exposed, tracheotomy made and etherization continued through the tracheotomy tube. Then both nerves were cut and their peripheral ends stimulated by induction currents. In most cases stimulation was carried on for about half an hour before, and for a similar period or longer after respiratory death. In three dogs the

[21] Joseph and Meltzer, *loc. cit.*

stimulations began only after death, and in fourteen they were carried on only before the death of the animal. In the antemortem period the individual stimulations were carried on for only thirty or forty seconds at a time, with intermissions of one minute or a little longer. In most cases only one vagus was stimulated at a time, alternating every few minutes between the right and left nerve. The strength of current which was employed in the antemortem period was just sufficient to stop the heart completely, or at least to slow its beats considerably. In the postmortem period a stronger current was frequently employed than the one used before death—the secondary coil often being kept even at zero. At that period also the stimulation was sometimes continuous and both vagi were often stimulated at the same time.

As we stated before, each experiment was accompanied by a control observation upon a normal animal of the same species. Such stimulation experiments were made on twenty-five dogs, eight cats and five rabbits. Five other stimulation experiments were made on atropinized cats.

Results.—We stated above that for good reasons we expected to find that such stimulations of the vagi as are capable of causing prolonged and repeated standstills of the heart would delay the development of rigor in that organ. We may state now, at the outset, that our experiments have demonstrated the reverse to be the case. *Not only do such stimulations not retard the development of cardiac rigor, but they definitely hasten it.* From the very beginning of this investigation and throughout the entire series, in almost every individual experiment, it was evident that those hearts whose vagi were stimulated developed rigor earlier than the hearts of the control animals. This alone, however, would not be conclusive. For, the time passing in the development of rigor varies in different animals even of the same species. Therefore, even if in a good many cases the stimulated heart developed rigor earlier than its accidental mate, still the stimulated heart might be actually retarded in the development of rigor, if it were compared with the hearts of other control animals. However, the conclusiveness of the result stated above is definitely demonstrated by a comparison of the sum total of the time consumed in the development of rigor in the hearts

of the control animals and of the animals whose vagi were stimulated. The following table (E) presents a comparison of the sum totals of the time elapsed (in minutes) between death of the animal and maximum rigor for each ventricle in the control and the stimulated hearts.

TABLE E.

Left Ventricle.

Number and Species of Animals.	A*		B†		Percentage of Reduction.
	Sum Total.	Average.	Sum Total.	Average.	
25 dogs.	5111	204	3565	142	30 per cent.
8 cats (non-atropined).	707	88	392	49	44 per cent.
5 rabbits.	461	92	288	57	38 per cent.

Right Ventricle.

24 dogs.	6239	260	4047	168	35 per cent.

* A. Control heart. † B. Heart whose vagi were stimulated.

This Table (E) illustrates strikingly the accelerating effect of vagus stimulation upon the development of rigor of the ventricles. For the left ventricle we have complete data for all three species of animals with which we experimented. All show a considerable reduction of the time between death and maximum rigor in the stimulated heart in comparison with that of the normal. In dogs the reduction is about thirty per cent.; in cats it amounts to almost forty-five per cent., and in rabbits to thirty-eight per cent. For the right ventricle we have complete data for twenty-four dogs. The reduction amounts here to thirty-five per cent. From the table it seems that, as far as the sum total of the three periods is concerned, there is practically no difference between the right and the left ventricle.

We reported in the first part of this paper[22] that prolonged etherization hastens the growth of rigor. Now, since all animals whose vagi were stimulated (Animals B) received ether for a longer period than most of the control animals, it could be claimed that the shortening of the time between death and maximum rigor in Animals B was not due to the stimulation of the vagi, but to the prolonged etherization. Against this we must refer to the other fact that the

[22] Joseph and Meltzer, *loc. cit.*

time elapsed between death and maximum rigor contains the periods of pulsation and relaxation, the duration of which is lengthened by prolonged etherization. Therefore a shortening of the time between death and maximum of rigor which could be caused by the hastening effect of ether upon the growth of rigor would be amply neutralized by the retarding effect of ether upon the two preceding periods. However, a more definite refutation of the before-mentioned claim will be found in Table F. It contains the figures for the average time elapsing between death and maximum of rigor in fourteen pairs of dogs in which the control and the experimental animals received ether for the same length of time.

TABLE F.

Left Ventricle.

Number and Species of Animals.	*A*		*B*		Percentage of Reduction.
	Sum Total.	Average.	Sum Total.	Average	
14 pairs of dogs	2,238	160	1,522	109	32 per cent.
		Right Ventricle.			
11 pairs of dogs.	2,641	240	1,597	145	40 per cent.

From the above Table (F) it is evident that the reduction in the time between death and maximum rigor in the stimulated hearts is due to nothing but the effects of vagus stimulation.

We shall now proceed to examine into the influence of stimulation of the vagi upon each of the three postmortem periods. For each period we shall give a seperate table containing the length of that period in both the control (A) and stimulated (B) animals. For each species only the average time (derived from the sum total of all the animals) will be given. The data for the left ventricle are complete. For the right ventricle we have data for dogs only. Table G contains the average length (in minutes) of the "pulsation period" of the left ventricle in A and B in all three species.

From Table G we learn that for dogs the pulsation time of the left ventricle in B is reduced with about the same amount as that of the entire time of development of rigor (Table E). In cats and rabbits, however, the pulsation period is greatly reduced. Al-

though in control rabbits the left ventricle continued beating after respiratory death longer than in the controls of the other two species, stimulation of the vagi cut this period down to less than one-third.

TABLE G.

Left Ventricle.

Number and Species of Animals.	*A*	*B*	Percentage of Reduction.
	Average (min.).	Average (min).	
25 pairs of dogs.	27	19	30 per cent.
19 pairs of dogs, with heart *in situ*.	10	7	30 per cent.
8 cats.	16	9	44 per cent.
5 rabbits.	32	10	69 per cent.

The following Table (H) presents the average length (in minutes) of the relaxation period of the left ventricle, *i. e.*, the period during which no part of the ventricle was either beating or in rigor in A and B of all three species.

TABLE H.

Left Ventricle.

Number and Species of Animals.	*A*	*B*	Percentage of Reduction A.
	Minutes.	Minutes.	
25 dogs.	28	—1	103.5 per cent.
19 dogs (with heart *in situ*).	23	8	65 per cent.
8 cats.	19	9	52 per cent.
3 rabbits.	23	9	61 per cent.

Table H demonstrates that the relaxation period is in all three species of animals greatly reduced through the stimulation of the pneumogastric nerves. By the relaxation period we designate, as stated above, the time during which on one hand all parts of the ventricle had stopped spontaneous contractions, and on the other hand no part had yet begun to show rigor. In the control animals this period was never absent, nor was it so very short. In the control dogs the period was, as a rule, more constant and a little longer than in the controls of the other species. In the stimulated dogs the period was greatly affected. In a number of animals there was

no relaxation period at all—the rigor setting in immediately on the cessation of all spontaneous motions in the ventricles. In others the rigor began in one part of the ventricle while there were still movements in another part of it. This was especially the case in the three dogs whose hearts were suspended in solutions of sodium chloride. In making up the averages, the time during which rigor was found in one part of a ventricle while another part was still beating, was included in the sum with a sign of "—," that is, this amount was deducted from the sum total. For all the twenty-five dogs the sum with a negative sign was larger than the one with a positive sign, so that the sum total had a negative sign. The average therefore amounted to — 1 and the percentage of reduction amounted to 103 per cent. When the relaxation time of the hearts which were suspended either in Ringer or in saline are excluded, and the sum made up alone of the dogs whose hearts remained in situ, the sum total becomes positive, although small. Even in these dogs the greater number had either no relaxation period, or else rigor and pulsations occurred in the same ventricle at the same time. It is a striking fact that in dogs the stimulation of the vagi, while affecting only moderately the pulsation period, nearly annihilates the period of relaxation in the left ventricle. This period is considerably shortened in cats and rabbits also. On opening the chest of one cat a minute after respiratory death, it occurred that in the stimulated heart the ventricles were already in complete rigor.

That the reduction in the periods of pulsation and relaxation was not due essentially to the postmortem stimulation of the vagi during these periods can be seen from the following short Table (I).

Table I contains the figures for the periods of pulsation and relaxation of the left ventricles in eight pairs of dogs, in which in B the stimulation of the vagi was carried out only antemortem.

TABLE I.

Left Ventricle.

Number and Species of Animals.	Period.	*A*	*B*	Percentage of Reduction.
8 pairs of dogs (Hearts of *B*'s were stimulated only antemortem — hearts *in situ*).	Pulsation	13 min.	8 min.	39 per cent.
	Relaxation	28 "	4.5 "	84 per cent.

This Table (I) shows that antemortem stimulations alone are certainly capable of reducing both periods quite strongly. The reduction is even somewhat greater than that shown by the entire 19 dogs with hearts *in situ* (which however includes three animals whose vagi were stimulated postmortem only).

In the following Table (J) figures are presented for the period of growth of rigor in the left ventricle, *i. e.*, the time elapsing between the beginning of rigor and the attainment of its maximum in A and B in all three species.

TABLE J.

Left Ventricle.

Number and Species of Animals.	*A*	*B*	Percentage of Reduction.
25 dogs.	145	119	18 per cent.
19 dogs (heart *in situ*).	138	98	29 per cent.
8 cats.	54	24	56 per cent.
4 rabbits.	45	26	42 per cent.

This Table (J) demonstrates that stimulation of the vagi accelerates also the growth of rigor, *i. e.*, its development from the beginning to the maximum. This effect seems to be exerted least in dogs and most in cats.

Table K contains the average figures for each of the three periods for the *right* ventricles of A and B in dogs.

TABLE K.

Right Ventricle.

Number and Species of Animals.	Period.	*A*	*B*	Percentage of Reduction?
25 dogs.	Pulsation.	45	37	18 per cent.
19 dogs (heart *in situ*).	"	30	33	—10 per cent.
25 dogs	Relaxation.	69	37	46 per cent.
18 dogs (heart *in situ*).	"	66	33	50 per cent.
25 dogs.	Growth of rigor.	143	101	29 per cent.
18 dogs (heart *in situ*).	"	153	92	40 per cent.

Table K demonstrates that also in the right ventricle in B the onset as well as the growth of rigor is accelerated. This is also true for the individual periods except for the period of pulsation in the dogs whose hearts were left *in situ*. That period is some-

what longer in B than in A. We shall not enter into a discussion upon this exception. The data for the right ventricle even in dogs were not exact enough to lay too much stress upon the absolute value of a single figure. Besides even in this exceptional case the sum of both first periods—that is the time elapsing between death and the beginning of rigor—is somewhat shorter in B than in A, which means that even in this case the stimulation of the vagi accelerated the onset of rigor of the right ventricle. Neither shall we enter into a discussion of some other minor points which this research brought to light. We wish, however, to mention the fact that in five pairs of cats which received enough atropin to eliminate the effect of the vagi the results in B differed little from those of A.

DISCUSSION.

This research establishes the fact that stimulation of the vagi before and after death with stimuli of sufficient strength to cause repeated and fairly prolonged standstills of the heart hasten the onset and development of rigor. The accelerating effect was manifestly present in all the three periods and in both ventricles. Leaving details aside, the chief result is that standstills of the heart and stimulation of the cardio-inhibitory nerves are favorable to the appearance of rigor in the cardiac muscle. This is surely a surprising revelation. In skeletal muscles increased motor activity hastens the development of rigor, which of course means at the same time that the decrease retards it. In the cardiac muscle the effect is just the reverse: considerable decrease of cardiac activity not only does not retard rigor, but hastens it. Furthermore, in skeletal muscle the hastening of the development of rigor is accomplished by motor impulses while in the cardiac muscle inhibitory impulses do the hastening.

Various hypotheses might be offered for the explanation of this phenomenon. It may be referred to the fact that the structure of cardiac muscle differs from that of the skeletal muscles and the assumption made that the functional difference with regard to the development of rigor is based upon this histological difference.

It may be referred further to the difference in the activity between the cardiac and the skeletal muscles: the heart is active all

the time and skeletal muscles are resting most of the time. And it may be assumed that any state of activity different from the prevailing one hastens the development of rigor. Therefore frequent and prolonged muscular contraction will hasten perceptibly the onset of rigor in skeletal muscles and prolonged and frequent standstills will hasten the development of the rigor in the heart.

Again it may be assumed that the hastening influence upon the skeletal muscles of a subminimum stimulation of the peripheral end of a motor nerve which is incapable of producing a contraction, has nothing to do with the motor impulse, but is due to some other effect which stimulation of the nerves produces, let us call it a trophic effect. Such trophic effects may be equally well produced by stimulation of nerves which otherwise are characterized by inhibitory effects. In the stimulation of the peripheral ends of the pneumogastric nerves the trophic effect favorable to the development of rigor may be so strong that it easily overcomes the unfavorable effect of the cardiac standstills which the stimulation causes at the same time.

Finally the following hypothesis may be offered. The frequent antemortem standstills of the heart favor a premature development of asphyetic conditions of the tissues, including that of the heart and it is this ante-mortem development of asphyxia of the heart muscle which favors the early development of rigor in the heart.

We shall not attempt to enter into a detailed discussion of the merits of these various hypotheses. But we wish to say that the last mentioned hypothesis appeals to us as the most plausible one. By the prolonged stoppages of the heart the coronary circulation suffers greatly. It has been known for a long time that impaired circulation hastens the onset of rigor. During the frequent standstills of the heart and the interrupted cardiac circulation, carbon dioxide and other deleterious substances accumulate within the heart muscle which may be capable of hastening its rigor. Probably more important is the gradual reduction in the content of oxygen, the presence of which was found by Fletcher[23] to be antagonistic to the development of rigor in frog muscles and was recently shown by

[23] Fletcher, *Jour. of Physiol.*, 1902, xxviii, 354.

Winterstein[24] to hold good also for mammalian muscles. Even if the inactivity of the heart does slow the consumption of the reserve oxygen, the frequent prolonged interruption in the supply of fresh oxygen will finally cause a serious deficit, so that at death there will remain in the tissues of the heart an insufficient amount of oxygen to hold back the onset of rigor for a normal period.

This hypothesis finds a support in other observations which we have made in this research. Along with the studies we made upon cardiac rigor, notes were frequently taken upon the development of rigor in the skeletal muscles of the same animals. We have referred above to these observations in comparing the heart with the skeletal muscles in regard to the time of onset of rigor. A review of these notes brings out further the fact that the onset of general rigor occurred in *B* earlier than in *A*. In twenty-one pairs of dogs it was found that the average time between death and the beginning of general rigor in *A* amounted to seventy minutes, while in *B* it was only forty-six minutes. In other words in animals in whom the heart was made to stop frequently by antemortem stimulations of the peripheral ends of the pneumogastric nerves, the rigor developed earlier than in animals whose vagi were not stimulated. For the present we see no other possible explanation of the connection between vagus stimulation and the development of general rigor, except upon the basis of the hypothesis that the frequent antemortem standstills of the heart favor a premature development of some state of asphyxia in the skeletal muscles and accelerate the onset of postmortem rigor in them.

SUMMARY.

In normal animals (dogs, cats and rabbits) which were killed by exsanguination, the beginning of rigor in practically all cases was separated from respiratory death by two well characterized periods: (1) a period of pulsation during which each ventricle still exhibited some spontaneous contractions, and (2) a period of relaxation during which the ventricles, while showing neither spontaneous contractions nor rigor, are more relaxed than in normal diastole, and during which the ventricles gradually lose their irritability.

[24] Winterstein, *Pflüger's Archiv*, 1907, cxvii, 225.

(The cardiac rigor or tonic contraction which was observed by recent investigators to set in immediately after death is an artificial phenomenon produced by filling the heart with saline and connecting it with a manometer.)

In the right ventricle both periods are longer than in the left ventricle, *i. e.,* rigor sets in later. Moreover the development of rigor from onset to maximum is also longer in the right ventricle than in the left, although here the difference is less striking.

The stopping of spontaneous beating, the disappearance of irritability, and the development of rigor, manifest in both ventricles a topographical progress from the base toward the apex, *i. e.,* the stoppage of beating, the disappearance of irritability and the setting in of rigor occur first at the uppermost part of the ventricle and last at the lowest point of the ventricle. It often occurred that rigor was already present in the basal part of a ventricle while the apex was still beating. It is probable also that the loss of vital activity and irritability and the development of rigor progress topographically from the endocardial to the epicardial surfaces.

Prolonged etherization retards the onset, but hastens the development of rigor; atropinization hastens both onset and development.

Repeated prolonged stoppages of the heart caused by antemortem (and postmortem) stimulations of the pneumogastric nerves *hasten* the onset as well as the development of rigor of the heart. All three periods are affected by the inhibitory influence of the stimulation, the period of relaxation, however, seems to be the one which is shortened most. The most probable interpretation of this phenomenon is the assumption that it is caused by an asphyxiation of the cardiac tissues. The stimulations of the pneumogastric nerves seem to hasten also the onset of the general rigor—probably this, too, is the result of some premature asphyxiation of the skeletal muscles.

THE ACTION OF GLANDULAR EXTRACTS UPON THE CONTRACTIONS OF THE UTERUS.[1]

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AND

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The uterine tissue consists of unstriped muscle fibre and ganglia of the sympathetic system imbedded in it. The sacral roots and pelvic nerve do not enter into the composition of the uterine plexus.

In the rabbit, irritation of the hypogastric nerve produces vaso-constriction and contraction of the uterus. In cats, stimulation of the hypogastric nerve produces at one time contraction and at other times relaxation of the uterus. Dixon[2] has shown that ergotoxin paralyzes the augmentor fibers and leaves the inhibitory fibers in the sympathetic intact. In the rabbit's uterus the augmentor fibers always predominate; hence an irritation always produces a rise of tonus in the uterus. After paralyzing the augmentor fibers with ergotoxin, adrenalin excites the inhibitory fibers and relaxes the uterus. In the unimpregnated uterus of the cat adrenalin relaxes the uterus, but with a pregnant uterus it causes contraction. However, Fardon[3] found that when nicotin is given first a contraction of the non-pregnant uterus is caused by adrenalin instead of the usual relaxation. Adrenalin is supposed to act on nerve endings; hence nicotin must act on these nerve terminals or on the muscle itself. This reversal of action of adrenalin by previous use of nicotin is not due to a paralysis of the inhibitory nerve endings, for if ergotoxin be added after the reversal has been produced, the action of adrenalin is once more reversed and produces inhibition.

[1] Received for publication November 23, 1908.

[2] Quoted by Fardon, *Bio-Chemical Journal*, 1908, iii, 405.

[3] *Bio-Chemical Journal*, 1908, iii, 405.

Our experiments number fifty-four and were made upon the uteri of cats and rabbits. We excised a small piece of the uterus, and one end was attached to a Porter heart-lever and the other end to the extremity of an L-shaped tube immersed in Ringer's solution. Oxygen was kept bubbling through the solution and the temperature was kept steadily at 37.5° C. by means of a water-bath and a small dental gas burner beneath the water-bath. The animals were first etherized, and then a small part of the uterus was excised. The powdered extracts were added directly to Ringer's solution. The oxygen supply was at a continuous rate of about two hundred bubbles a minute. Two hundred cubic centimeters of Ringer's solution were used.

Normal Movements.—In the pregnant uterus the movements are more pronounced than in the unimpregnated one. The non-impregnated uterus of young animals does not give curves of any

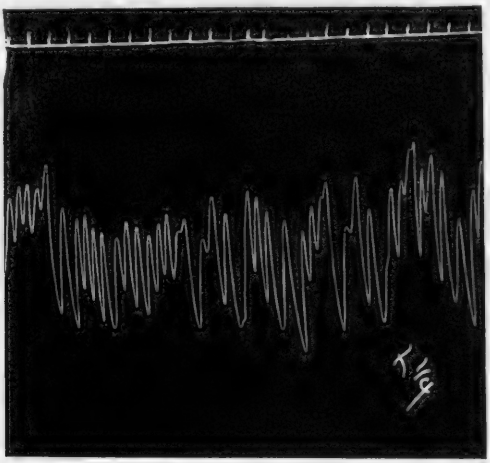

FIG. 1. Normal uterine contractions. Effect of mammary gland extract on uterine contraction.

extent, and adding adrenalin, a sympathetic nerve stimulant, does not seem to better the results. The uterine contractions normally show considerable variation in regard to chemical stimuli. The animal extracts used were those of Armour & Co., except where otherwise stated.

Mammary Gland.—Extract of mammary gland in doses of ⅛ to 1 grain augmented the uterine contractions and increased the tonus (Figs. 1 and 2). Large doses relaxed the uterus, diminished in extent and slowed contractions.

Prostate.—The prostate in doses of ¼ to 1 grain increased the extent of the uterine contractions and increased the tonus of the uterus. In the cat the uterus took on after one grain of the extract

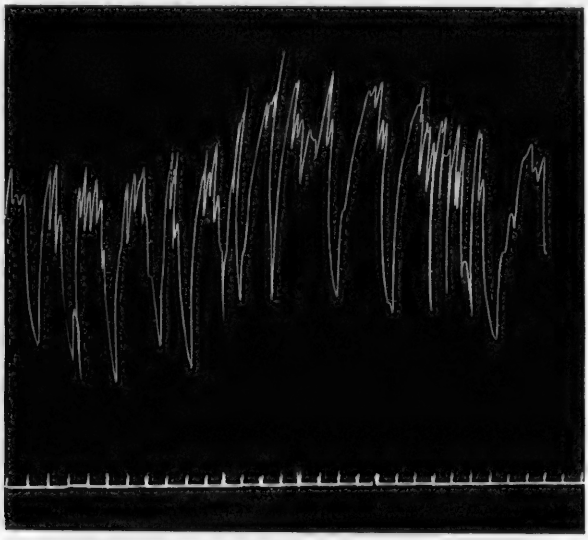

FIG. 2. After effect of ¼ grain of extract of mammary gland (same experiment as Fig. 1). Read from left to right. Time marks every 5 seconds.

a series of short and frequent contractions, followed by contractions much larger than normal (Figs. 3 and 4). Tonus was primarily decreased after a dose of one grain.

Spleen.—In doses of ¼ to ½ grain, splenic extract increased slightly the uterine contractions; there was no effect on tonus.

Testicle.—In doses of ¼ to ½ grain, testicular extract slightly increased the extent of uterine contractions; there was increased tonus.

Parotid Gland.—In doses of ⅛ to ½ grain, parotid extract

slightly increased the extent of the uterine contractions; there was increased tonus.

Pancreas.—In doses of 1/8 to 1/4 grain, pancreatic extract increased the extent of the uterine contractions and slightly augmented the tonus.

Pituitary Body.—In doses of 1/8 to 1 grain, pituitary extract increased the extent of the contractions of the uterus and the tonus. This effect has been noticed by Shäfer.

Thymus.—In doses of 1/8 to 1 grain, extract of thymus slightly increased the extent of uterine contractions; it increased tonus.

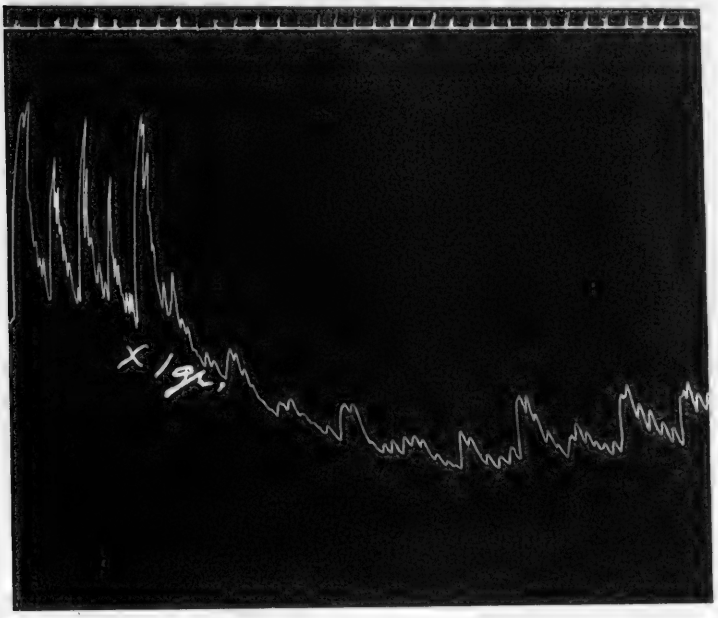

FIG. 3. Primary effect of prostate on uterine contractions.

Iodothyrin.—This agent in doses of 1/8 to 1/2 grain increased the extent of uterine contractions and the tonus.

Spermine (Poehl).—In one- to five-drop doses, spermina increased the extent of uterine contractions; there was slight increase of tonus.

Ovary.—In doses of ⅛ to 1 grain, extract of ovary augmented the extent of the uterine contractions; there was increased tonus.

Dried Brain.—In doses of ⅛ to 1 grain, dried brain gave a marked increase of extent of uterine contraction and increased tonus.

Adrenal.—This agent, in one- to two-drop doses, always caused a marked contraction of the uterus; it also increased the tonus in

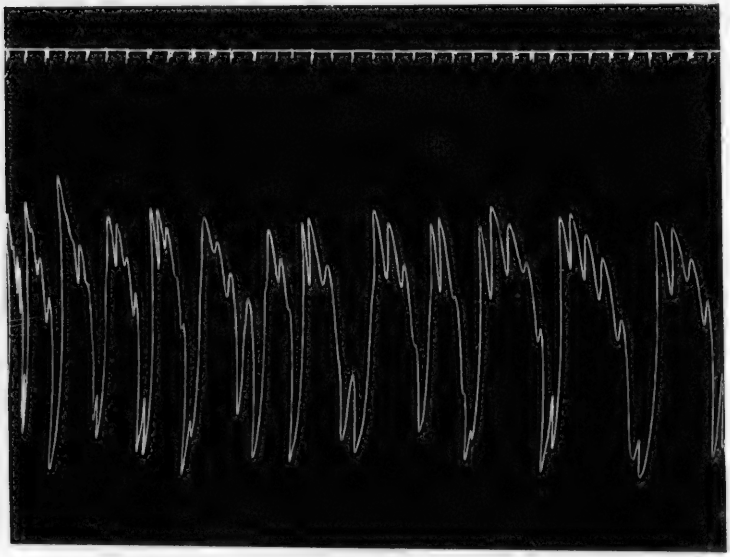

FIG. 4. After effect of prostate on uterine contractions in the same experiment.

the rabbit. In the non-pregnant cat we found, as Fardon did, that it relaxed the uterus and the contractions ceased or nearly ceased.

Of all the extracts those of the mammary gland and the prostate had the most energetic action on the uterus. Starling[4] has shown that the embryo develops a chemical messenger (harmone) to stimulate the cells of the mammary gland. It seems that mammary glands secrete or contain a substance which stimulates the muscle cells of the uterus.

[4] *Ergebnisse der Physiologie,* 1906, v, 686.

THE PRESENCE OF A WEAK HEMOLYSIN IN THE HOOK WORM AND ITS RELATION TO THE ANEMIA OF UNCINARIASIS.[1]

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The anemia which is usually present in uncinariasis has been a favorite topic of discussion by all writers on the subject, and many explanations have been advanced. Perhaps the most popular theory depends on the assumption that the hook worm secretes some hemolytic principle which is absorbed by the host and destroys the red cells. The object of this paper is to show that the hook worm of man does contain a very weak hemolytic agent, active *in vitro*, but that this hemolysin is demonstrable only in concentrated extracts of the hook worm, and could have no relation to the secondary anemia of the host. The hemolysis takes place slowly, very little being recorded in six hours, and the readings were usually made after twenty hours. The hemolysin is present in all parts of the worm, and probably is related to its intestinal canal—a fact which excludes the possibility of any active hemolysin being secreted by the cephalic glands of the hook worm. The following fact goes to show that some hemolysin is present in the alimentary tract of the worm. Hook worms containing fresh blood removed from the mucosa, washed and placed in citrated saline, at once begin to pour out the intestinal contents from mouth and anus. This material is made up of cell detritus, intact red blood cells and *blood-stained* fluid. That we see many intact red blood corpuscles in the material passed out of the anus, speaks for a rapid ingestion and a weak hemolytic factor.

In a recent communication (8) on "Uncinariasis in Panama" I have reviewed the anatomical findings in a series of autopsies, recorded the frequency of hook worm infection, and noted some

[1] Received for publication December 12, 1908.

facts relating to the anemia of this disease. It has been shown that the hook worms ingest blood and epithelial cells from the mucosa and presumably digest both. Evidence has been brought forward to indicate that the anemia is due to two factors at least: (1) direct loss of blood through activity of the parasites; (2) a diffuse inflammation of the mucosa and submucosa of the jejunum caused by the bites of hook worms which damage the mucosa and give entrance to the intestinal bacteria. Attention has been called to the *"blood cysts"* which often contain hook worms. The lesion is due to erosion of a vessel by the hook worm in the submucosa giving rise to a definite hematoma. The hook worm may live in this blood clot for a few days as shown by the number and character of wandering cells in the margin of the clot. The worms when removed are always active, and show no signs of injury due to their stay in the blood of the host. Here we have an ideal experiment performed by the hook worm—the formation of a small sac of blood in the tissues of the host with an included parasite and all conditions favorable for the action of any hemolytic agent—yet we find very little solution of the red blood corpuscles. Study of such tissues shows many well-preserved red blood cells in all parts of the blood cyst, and very little evidence of hemolysis. It seems that this observation alone is sufficient to exclude the presence of any powerful hemolysin in the hook worms, and is a link in the chain of evidence that the hook worm does not secrete a hemolysin, which is absorbed by the host and gives rise to the secondary anemia.

It seems probable from this series of observations that the weak hemolysin found in the hook worm is present in the digestive tract, and possibly related to its digestion of the host's blood—a part of the parasite's food. The hook worms used in these experiments were taken from the intestinal mucosa at autopsy and used while actively motile: such material seems to be more valuable than that obtained from stools after the exhibition of thymol. If we compare the experiments in which human hook worms were used, with those in which dog hook worms were studied, we find that the results are similar—that both types of hook worm act on the blood of man and the dog. Parallel experiments with the whip worm and round worm of man act as excellent controls and show that

concentrated extracts of whip worms may be faintly hemolytic to some bloods and not to others. Emulsions of *Ascaris lumbricoides* are never hemolytic even when the worms are unwashed and carry numbers of intestinal bacteria into the mixtures.

Various experiments have been recorded to show the presence or absence of a hemolysin in various intestinal parasites. Loeb and Smith, working with *Ankylostomum caninum,* demonstrated the presence of a coagulation inhibiting substance in the cephalic glands and noted that in their mixtures there was no hemolysis in seventeen hours. Their method was very similar to that used in these experiments, but their emulsions were very much more dilute: about six or eight worms to one cubic centimeter of extract. In these experiments from twenty to fifty worms to one cubic centimeter of extract were used and the incubation was longer. Their experiments show how weak is this hemolytic factor.

Weinberg, working with *Sclerostomum equinum,* demonstrates a hemolysin which is active toward the blood of the horse and various other animals. It is more marked in the head of the worm, but is present in the digestive tract as well. It is quite resistant to heat and is easily soluble in saline. Other intestinal parasites of the horse show no such hemolysin (*Oxyuris equi, Ascaris megalocephala* and *Tenia plicata*).

Preti has studied the Old World hook worm (*Ankylostomum duodenale*) and states that he finds a hemolytic substance. This is not soluble in saline, but easily soluble in ether and alcohol. It is not destroyed by boiling for three hours. Trypsin frees the hemolysin and makes it soluble in water. In this preliminary communication he gives no information concerning his methods and controls, nor does he apply his methods to other intestinal worms. He makes no statement as to the rapidity of the hemolysis. He believes that the hemolytic substance is related to the group of lipoids and is very similar to the hemolysin isolated from *Bothriocephalus latus* by Tallqvist.

Many other writers (Ashford and King, Baker) cite the pigmentation of the liver and spleen, the hyperplasia of the marrow, and the advanced anemia as conclusive evidence of some hemolytic substance produced by the hook worms and absorbed by the host.

All these changes are found in secondary anemias caused, for example, by any long-continued bacterial infection of low grade, and evidence has been brought to show that there is in many cases of uncinariasis just such a low grade bacterial infection with subacute inflammation in the mucosa or submucosa. The increase of eosinophiles in the marrow and in the tissues close to the parasites is not peculiar to this disease, and is no argument in favor of a hemolysin.

HOOK WORMS OF MAN.

The first three experiments (Charts I, II, and III) can be compared as the same emulsions were used in each. Emulsion (1) of seventy New World female hook worms was made up in 3.5 cubic centimeters saline. Some of the worms were dead and the emulsion had a slight putrefactive odor. All emulsions were used after standing for thirty minutes, which gave opportunity for all particles to sediment out, leaving an opalescent supernatant fluid. Emulsion (2) of sixty Old World hook worms (male and female) was made up in 3.5 cubic centimeters saline; all worms were alive. These worms together are of greater bulk than those in Emulsion (1), and there is no odor. Emulsion (3) of thirty New World male hook worms was made up in 3.5 cubic centimeters saline.

CHART I.

Citrated Blood in Uniform Dilution (Human).

Hook worm emulsions.	Black male, 20 years.							White male, 25 years.							
(1) In 3.5 c.c. saline. 70 new world females.	4				4			4					4		
(2) In 3.5 c.c. saline. 60 old world worms.		4	10			4			1	4	15			4	
(3) In 3.5 c.c. saline. 30 new world males.				4			4					4			4
Hemolysis.	1	2.5	3.5	1	1	1.5	1	2	0.5	2	3	2	2	2.5	2
Readings after 22 hours.					Incubated 6 hours at 38° C.								Incubated 6 hours at 38° C.		

Figures indicate number of drops of emulsion added to citrated blood.

The above charts (I, II and III) show that there is a weak hemolytic agent present in both Old and New World varieties of hook worms—in both males and females. It acts slowly and there is scarcely any hemolysis noted in six hours. Incubation at 38° C. has the slightest or no effect. The hemolytic agent acts on the blood of man and of the dog and rat.

CHART II.

Human Citrated Blood.

Hook worm emulsions (as in Chart I).	White male, 45 years.											
(1) 70 new world female worms.	4	15	15 sed.	23								
(2) 60 old world hook worms.					4	15	15 sed.	24				Controls.
(3) 30 new world male worms.									5	15	25	
Hemolysis.	1	1	1	2	0.5	1	3	2	0.5	0.5	1	0

Readings after 22 hrs.; (sed.) indicates the fine sediment which settles out from any emulsion after standing.

CHART III.

Animal Citrated Blood.

Hook worm emulsions (as in Chart I.).	Mongrel active pup.								Adult white rat.							
(1) 70 new world females in 3.5 c.c. saline.	4				4			Controls.	1	4	15			4		
(2) 60 old world worms in 3.5 c.c. saline.		4				4						4			4	
(3) 30 new world males in 3.5 c.c. saline.			4	15			4						4			4
Hemolysis.	1	1.5	1	1.5	1	1	1	0	0.5	1	2	1	0.5	1	2	0.5
Readings after 22 hours.	Incubated 6 hrs. at 38° C.															

The emulsions used in Experiment 4 (Chart IV) were made as usual. The active hook worms were cut in two, leaving about one-fifth of the worm in the head end. The œsophagus of the worm is easily visible, and the cut was made about 0.5–1 mm. posterior to the œsophagus. Emulsions were placed in ice box over night and the clear supernatant fluid used, except where marked (sed.), indicating the use of the finely granular sediment.

Chart IV shows that the hemolytic factor is present in all parts of the hook worm, in the head end as well as in the body, but more marked in the larger body portion. The clear supernatant fluid is hemolytic as well as the granular sediment of the emulsions. The whip worm emulsion is not hemolytic to the blood of these two persons.

Experiment 5.—The worms were cut in two exactly as in Experiment 4. The worms were all alive and active when used except in (5) where they had been dead for twenty-four hours. Emulsion (5) of forty dead hook worms contained both Old and New World types.

CHART IV.

Citrated Blood in Uniform Dilution (Human).

Hook worm emulsions.	White male, 45 years.												Black male, 20 years.			
(1) Bodies of 25 large old world hook worms in 1.5 c.c. saline.	10				10				10 sed.				10			
(2) Heads of 25 large old world hook worms in 1 c.c. saline.		10		Control.		10		Control.		10 sed.				10		Control.
(3) Emulsion of 3 large whip worms in 1.5 c.c. saline.			10				10				10 sed.				10	
Hemolysis.	3.5	1.5	1	1	2	trace	0	0	2	0.5	0		1	1	0	0
Readings after 22 hrs.	Incubated at 38° C. for 22 hrs.								(Sed.) — Indicates the use of finely granular sediment in the emulsions.							

Figures indicate number of drops of emulsion added to citrated blood.

The emulsions were all placed on ice over night and when used all were odorless except (5) which showed a strong putrefactive odor. The supernatant fluid was slightly opalescent. The sediment was abundant and rich in sand which had been added in greater amounts than usual.

CHART V.

Citrated Blood in Uniform Dilution.

Hook worm emulsions.	White male, 25 years.												Small anemic pup.					
(1) 82 new world female (bodies) in 2.5 c.c. saline.	10						10						10					
(2) 82 new world females (heads) in 2 c.c. saline.		10						10						10				
(3) 77 new world males in 2.3 c.c. saline.			10			Control.			10			Control.			10			Control.
(4) 17 old world worms in 2 c.c. saline.				10						10						10		
(5) 40 dead hook worms in 2 c.c. saline.					10						10						10	
Hemolysis.	0.5	3	0.5	1	3	trace	0.5	2.5	0.5	3	3	0.5	3	3	3	4	5	1
Readings after 24 hrs.	Supernatant fluid.						Sediment used was very rich in sand.											

Figures indicate number of drops of emulsion added to citrated blood.

Chart V shows that the hemolytic agent is present in both body and head parts of the hook worms (see Chart IV) in both males and females. The

supernatant fluid and sediment are both hemolytic. The dead worms are slightly more hemolytic than the live ones, and this is possibly due to the putrefactive products. Emulsions of human hook worms will hemolyse human and dog blood. The pup's blood used in this experiment was always very sensitive to any hemolytic agent.

Experiments 6 and 7 are very similar. Emulsion (1) was made as usual from Old World hook worms (males and females); there was greater concentration in Experiment 6. Pancreatic extract (2) was made as follows: a dog's pancreas was freed from fat, finely divided and ground up with sand to a thick paste; 12 grms. of this material was placed in a flask and 70 c.c. of distilled water and 3 c.c. of chloroform were added. The mixture was incubated for twenty-four hours at 38° C. This fluid digested coagulated egg albumen, but was not very active at room temperature. Solution of sodium carbonate (3) was quite dilute, but its strength was not accurately determined; it gave a definite blue reaction on litmus.

CHART VI.

Human Citrated Blood.

	White male, 18 years.								
(1) 30 old world hook worms in 1.4 c.c. saline.	5	10	15 sed.	5	5	5			Control.
(2) Pancreatic extract (dog).				1	5			5	
(3) Sodium carbonate in dilute solution.						5	5		
Hemolysis.	1	1.5	3	1.5	1.5	1	0	2	faint trace.
Readings after 22 hours.									

Figures indicate number of drops of emulsion added to citrated blood.

CHART VII.

Human Citrated Blood.

	White male, 22 years.								
(1) 44 old world hook worms in 2.5 c.c. saline.	5	10	15	Control.	10 sed.	15	15	15	Control.
(2) Pancreatic extract (dog).							10		
(3) Sodium carbonate in dilute solution.								10	
Hemolysis.	0.5	1	1	0	3	3	2	2	faint trace.
Readings after 18 hours.					Incubated at 36° C. for 18 hrs.				

The Charts VI and VII show that the hemolytic factor present in the Old World hook worm is uninfluenced by pancreatic extract or a weak solution of sodium carbonate. Incubation increases the hemolysis in small degree.

Experiments 8 and 9 must be compared as they both show the results of boiling, which destroys the hemolytic factor both in the human and the dog's hook worm.

Experiment 8.—Emulsion of 90 hook worms (Old and New World varieties) was made up in 3 c.c. of saline and divided into the three equal parts indicated in Chart VIII.

CHART VIII.

Citrated Blood in Uniform Dilution.

Emulsion of 90 hook worms in 3 c. c. saline.	White male, 18 years.														Strong active pup.				
(1) 1 c.c. used fresh.	10			15	5 sed.				5						10			10 sed.	
(2) 1 c.c. in boiling water bath for 15 minutes.		10				10 sed.		Controls.		5		10 sed.		Controls.		10			Controls.
(3) 1 c.c. in boiling water bath for 60 minutes.			10				10 sed.				5		10 sed.				8		
Hemolysis.	1	0	0	2	2	0	0	0	1	0	0	0	0	0	1	0	0	1	0
Reading in 30 hrs.									Incubated 20 hrs. at 38° C.										

Figures indicate number of drops of emulsion added to citrated blood.

Chart VIII shows that boiling for fifteen minutes or over completely destroys the hemolytic agent. The hemolytic factor present in the human hook worm acts on dog's as well as on human blood (see Chart IX).

CHART IX.

Citrated Blood in Uniform Dilution.

Emulsion of 60 dog hook worms in 2 c.c. saline.	White male, 21 years.									Small anemic pup.					
(1) 1 c.c. used fresh.	10	Control.		10	10*	15*	Control.	10	10	Control.	10		10[1]	15[1]	Control.
(2) 1 c.c. placed in boiling water bath for 3 minutes.			10									10			
Hemolysis.	0.5	0	0	2	0.5	1.5	0	2	0	0	2	0.5	2	2	0
Reading after 20 hrs.			Incubated at 36° for 16 hrs.								Incubated at 36° C. for 16 hrs.				

Figures indicate number of drops of emulsion added to citrated blood.

* Sediment taken up in 1 c.c. saline, well mixed and used at once.

Hook Worm of Dog.—The experiments with *Ankylostomum caninum* were performed in precisely the same manner as were those with the hook worm of man, and usually at the same time. All conditions were equal and we may compare the results which are very similar.

Experiment 9 (compare Chart VIII).—Emulsion of 60 dog hook worms (males and females in about equal numbers) made up in 2 c.c. saline and divided into equal parts: (1) used fresh. (2) 1 c.c. of the turbid fluid placed in a boiling water bath for three minutes. Solution was placed on ice for one hour and then used.

Chart IX shows that the dog hook worm contains a hemolytic factor which is active towards human as well as dog blood. Boiling for 3 minutes destroys practically all of the hemolysin. The sediment taken up in saline and used at once shows that not all the hemolytic agent is extracted in the first emulsion.

Experiment 10 (compare Experiments 4 and 5).—One hundred hook worms (*Ankylostomum caninum*) were cut in two as in Experiment 4. The "head end" contains about one-fifth of the worm, including the cephalic glands, and the "body" contains the remaining four-fifths of the worm. Sodium carbonate solution (3) is that used in Experiments 6 and 7.

CHART X.

Citrated Blood in Uniform Dilution.

	Male adult dog.					Negro male, 25 years.								White male, 45 years.							
(1) 100 *heads* in 2 c.c. saline. Dog hook worms.	3		10			3		10		10				3		10		10			
(2) 100 *bodies* in 2.5 c.c. saline. Dog hook worms.		3		10	Controls.		3		10		10		Controls.		3		10		10		Controls.
(3) Sodium carbonate weak solution.										5	5	5						5	5	5	
Hemolysis.	trace	0.5	0.5	1	0	0	0.5	0.5	0.5	0.5	0.5	0	0	0	0	0.5	0.5	trace	0.5	0	0
Reading after 16 hrs.	No incubation.																				

CHART XI.

Human Citrated Blood.

	White male, 21 years.						
(1) Emulsion of 5 whip worms in 1.2 c.c. saline.	5	5	10	5 sed.	3 sed.		Control.
(2) Weak solution of sodium carbonate.				5	6	6	
Hemolysis.	0	1	1	0.5	0	0	trace
Readings in 18 hours.	All mixtures faintly alkaline to litmus.						

Figures indicate number of drops of emulsion added to citrated blood.

Chart X shows that Ancylostomum caninum, like the human hook worm, contains a hemolytic agent in all parts of its body, which is active for the blood of man and of the dog. Sodium carbonate in weak solution has no effect on the hemolysis. The larger body fraction seems to have a little more activity than the head fraction.

Whip Worms of Man.—Experiments 11 and 12 are very similar. Emulsion (1) was made with large active whip worms.

CHART XII.

Human Citrated Blood.

	White male, 22 years.					
(1) Emulsion of 5 large whip worms in 1.5 c.c. saline.	10	15	15			Control.
(2) Sodium carbonate dilute solution.			10	20		
Hemolysis.	0.5	2	1.5	0		0
Reading after 22 hours.		Incubated at 36° C.				

Charts XI and XII show that concentrated saline extracts of the human whip worm may contain a very weak hemolytic agent active toward the blood of some persons. It is uninfluenced by a weak solution of sodium carbonate.

CHART XIII.

Human Citrated Blood.

	White male, 18 years.						
Emulsion of 8 whip worms in 2 c.c. saline.	10	10	Control.	10	10	10	Control.
Weak solution of sodium carbonate.					5	5	
Hemolysis.	0.5	1	0	3	3	3	0
Readings in 18 hours.	Incubated at 37° C. for 18 hours.						

CHART XIV.

Human Citrated Blood.

	White human, 25 years.											
Emulsion of 10 whip worms in 2 c.c. saline.	5	10	15	10 sed.	15 sed.	Control.	5	10	15	10 sed.	15 sed.	Control.
Hemolysis.	0	0	0	0	0	0	0	0	0	0	0	0
Readings after 24 hours.	Incubated at 38° C. for 7 hours.											

Experiments 13 and 14 may be contrasted, as the emulsions are very similar, yet the blood of one case is hemolyzed and the blood of the other resists hemolysis. The blood used in Experiment 14 was always resistant to hemolytic agents.

Experiments 11–14 (see also Experiment 4) indicate that the human whip worm may contain a weak hemolytic agent, active toward some solutions of blood and not towards others. This action may be increased by incubation, but is unaffected by sodium carbonate in weak solution.

CHART XV.

Human Citrated Blood.

Emulsions of female *Ascaris lumbricoides.*	White male, 45 years.													
(1) Head — 1 cm. in 2 c.c. saline.	5	10					Control.	15 sed.			15			Control.
(2) Body — 1 cm. in 2.5 c.c. saline.			5	10					15 sed.			15		
(3) Tail — 1 cm. in 2.5 saline.					5	10				15 sed.			15	
Hemolysis.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Readings after 24 hours.											Incubated at 36° C. for 24 hours.			

Figures indicate number of drops of emulsion added to citrated blood.

CHART XVI.

Human Citrated Blood.

Emulsions of female *Ascaris lumbricoides.*	Black male, 25 years					
(1) Head end (1 cm.) *not* washed in 2.5 c.c. saline.	51	15 sed.				Control.
(2) Tail end (1 cm.) washed in 2.5 c.c. saline.			10	15		
(3) Body (1 cm.) washed, incubated — 45 mm.					15	
Hemolysis.	0	0	0	0	0	0
Readings in 27 hours.						

Round Worm of Man.—The last two experiments (15 and 16) are very much alike in all respects. In Experiment 15 a medium-sized female, *Ascaris lumbricoides,* was thoroughly washed in water and saline. For Emulsion (1) the head end was cut off (1 cm. in length) and ground up with sand and saline added

to 2 c.c. For Emulsion (2) a piece was cut out of the middle of the worm, a little less than 1 cm. in length, and made up in 2.5 c.c. saline. For Emulsion (3) about 1 cm. of the tail end was made up in 2.5 c.c. saline.

Experiment 16 was identical with Experiment 15 except that the head end (1) was cut off before the worm was washed and this emulsion was rich in intestinal bacteria. Emulsion (3) of body was incubated at 38° C. for forty-five minutes before use.

Charts XV and XVI show that the round worm of man (Ascaris lumbricoides) contains no hemolytic agent in any portion of its body.

Method.

Emulsions in all cases were made as follows: the hook worms were washed thoroughly in running water; then in normal saline solution. The living worms alone were used unless otherwise stated. In almost all cases the worms were studied and identified, as indicated in each experiment. The worms were ground up in a small mortar with clean, white sand and a few drops of saline added; then the grinding was continued until a thin, uniform, gray paste was formed. To this was added a measured quantity of 0.7 per cent. saline solution (1–5 c.c.) as is indicated in the charts. These turbid mixtures were placed on ice for a varying length of time. Unless otherwise stated the supernatant fluid which was usually opalescent was added to the blood.

The citrated blood was prepared in the same manner in all cases. Small test tubes usually used in hemolytic work, were half filled with citrated saline solution (7 grms. sodium chloride and 15 grms. sodium citrate dissolved in 1 liter of distilled water). To this was added three large drops of blood usually obtained from the ear thoroughly cleansed with alcohol. This gave a dilution of blood best suited for the work—about one part blood to eight or ten parts citrated saline. After addition of the emulsion by a long dropper, the tubes were inverted several times to get a uniform mixture. The majority of the tubes were left at room temperature which was quite constant at Ancon (75°–85° F.). Other tubes were incubated in the thermostat. Hemolysis was estimated by the writer in all cases and is expressed arbitrarily by numbers, considering complete hemolysis as 10. The estimation was done carefully, and we feel that the relative values or amounts of hemolysis are fairly accurate. The charts are all made on the same plan as Chart I. The citrated blood is of constant dilution—human or animal as indicated at the top of the chart. The figures in the upper columns indicate the number of drops of the emulsion added to the citrated blood, and the resulting hemolysis is expressed by figures in the bottom column.

SUMMARY.

1. The hook worm of man—both the Old and New World types—contains a weak hemolytic agent active *in vitro*. It is soluble in salt solution, is easily destroyed by heat and acts slowly.

2. The hemolysin is present in all parts of the worm and probably is associated with the intestinal tract.

3. The hook worm of the dog contains a similar hemolysin.

4. These hemolysins are not specific, but will act on human blood as well as on that of the dog and rat. They are only demonstrable in concentrated extracts.

5. Concentrated extracts of the human whip worm may be hemolytic to some bloods.

6. The round worm of man contains no hemolytic principle in any part of its body.

7. It seems very unlikely that this weak hemolysin found in the hook worm can have any relation to the anemia of uncinariasis.

8. Study of the "blood cysts" in the human intestine shows that the hook worm may live in a small amount of the host's blood for days without causing any marked hemolysis.

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AN EXPERIMENTAL STUDY OF THE METABOLISM AND PATHOLOGY OF DELAYED CHLOROFORM POISONING.[1]

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Plates VIII–X.

In the course of some investigations which have occupied our attention during the past four years, we used prolonged and repeated inhalations of chloroform in an attempt to diminish the oxidative power of the cells and thus to inhibit or prevent the transformation of certain substances with which we were experimenting. We found, however, that a number of control animals which received chloroform alone, were profoundly affected and died after a lapse of hours or days after their return to consciousness, and that they all exhibited severe parenchymatous lesions, especially in the liver. In other words, we produced typical delayed chloroform poisoning. It appeared to us advisable in view of the great importance attaching to delayed poisoning to study the metabolic changes occurring as a result of this, and in comparison with the pathological lesions produced. Dogs, upon which metabolic experiments are most easily carried out, can also regularly be so anæsthetized as to cause their death many hours after the anæsthesia.

The conception of delayed chloroform poisoning has been of slow development. Although Casper (1) and Langenbeck (2), in 1850, and Mouat (3), in 1856, spoke of a postponed fatal effect of chloroform, it was not until more than thirty years later that the condition began to be generally recognized and accepted. Thiem and Fischer (4), in 1890, reported a fatal case due, they believed, to the delayed effect of chloroform. In the following year Bastianelli (5) reported three cases, and in 1892 Fränkel reported several more. Thereafter,

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reports appeared in rapid succession by Ambrosius, Guthrie, Bandler, Marthen, Heintz, Steinthal, etc. In England, Guthrie's paper first called attention to the condition, and in this country cases have been reported by Brewer, Bevan and Favill, Holmes, Cushing, Ballin, Wells and others. It is undoubtedly true that some deaths have been reported as due to chloroform that could, with greater probability, be referred to some other cause, such as the original disease or antiseptics, but this does not effect the validity of the majority of reported cases.

Nothnagel (6), in 1866, was the first to study the lesions produced by chloroform in animals. In his experiments chloroform was given by mouth and subcutaneously, and not by inhalation. The amount employed was, moreover, so large that most of the animals died in a few hours, or he killed them at that time. Nevertheless, he produced fatty changes in the heart, liver and kidneys.

The first attempt to explain delayed poisoning was made in 1883 by Ungar with the help of Junkers (7). Ungar had been impressed with the occasional, unfavorable outcome after operation when everything apparently had been progressing favorably until a short time before the patient's death. He appreciated the great difficulties in the way of separating symptoms and pathological changes due to chloroform from those due to the primary disease or injury necessitating operation, and therefore decided to use animals. His experiments were carefully planned and carried out, and his results of great importance. He attempted first to repeat Nothnagel's experiments, but could obtain no satisfactory results. He then administered chloroform by inhalation to rabbits and dogs. Rabbits were difficult to use on account of their great susceptibility to chloroform during its prolonged administration, but in dogs he was able to produce great fatty changes in the liver and heart and less in the kidneys. He observed that prolonged administration was necessary and that the lesions were much more severe when repeated inhalations were employed. Unlike most subsequent investigators, he let his animals die and so obtained more severe lesions than have those who after twenty-four, forty-eight or more hours killed their animals. Like many who followed him, it is evident that he began with a preconceived idea as to the organ most probably responsible for death, and wrote his descriptions of lesions from that standpoint. He found great change in the cardiac muscle and

inclined to this as the cause of death. From his description of the appearance of the livers, both macroscopical and microscopical, it is apparent, however, that in them very severe lesions were present. He says that the center of the lobules was made up of fatty detritus only; the cell outline was indistinguishable.

In 1889, Ostertag (8) and Strassmann (9) independently confirmed Ungar's findings. Ostertag experimented on many different animals: rabbits, guinea-pigs, pigeons, rats, cats and dogs, and obtained fatty changes in all. Some of his animals died of late poisoning, but he was unable to account for this, and recognized in them no necrotic lesions. He made the important observation that dogs exhibited great differences in resistance to the chloroform.

Thiem and Fischer (10), in 1890, used rabbits and dogs and produced marked fatty changes, especially in the livers. In 1896 Bandler (11) and Heintz (12), independently, recognized necrosis in the livers of fatal cases and produced necrotic lesions in the livers of dogs and rabbits. In the same year Marthen (13) reported a fatal case with liver necrosis and Ajello (14) caused experimental necrosis in the livers of animals. From this year dates the recognition of necrosis as the most important of the changes produced by chloroform. In fact, hardly an autopsy since that time has been reported that has not shown necrosis, and in many it has been so marked that the cases have been denominated acute yellow atrophy.

Lengemann (15), in 1900, was unable to produce delayed chloroform poisoning in a dog by repeated inhalations when the amount of chloroform was carefully measured and just enough given to produce anæsthesia and no more. Other experimenters, whose work should be mentioned, are Stiles and McDonald (16) and Müller (17). There has been practically complete agreement among all writers during the last ten years that the most extreme lesions are produced in the liver, and that here is to be found the chief cause of death. Offergeld (18) alone dissents from this view and thinks the kidneys are the organs chiefly involved.

There is no record in the literature, so far as we know, of a study of the metabolism in animals in which such a condition of delayed chloroform poisoning has been experimentally induced. The metabolic effects of chloroform given by mouth, by subcutaneous injection or by inhalation have been widely studied, but in every case, normal, uneventful recovery apparently occurred. Strassmann (19) made estimations of the urinary nitrogen before and after chloroform inhalation in an effort to determine whether the fatty change in the liver was an infiltration or degeneration. He concluded that fatty degeneration had occurred because of a marked increase in the nitrogen excreted. This increase amounted to twenty per cent. on the day on which chloroform was given and continued

for the two following days. In Salkowski's (20) experiment a dog in nitrogenous equilibrium was given fifteen cubic centimeters of chloroform in two hundred cubic centimeters of water on three successive days. Narcosis was not produced but the nitrogen elimination increased, the increase being noticeable on the day following the end of the chloroform period. Taniguti (21) under Salkowski's direction showed in one experiment that inhalation anæsthesia by chloroform caused an increased nitrogen excretion lasting for two days after the inhalation. He also noted an increased elimination of phosphoric acid which was not, however, parallel to the elimination of nitrogen. Kast and Mester (22) examined changes in the sulphur excretion after anæsthesia with chloroform in human subjects. The earlier results by Kast (23), which showed a marked increase in the elimination of chlorine in the urine of fasting dogs anæsthetized with chloroform, led them to the belief that metabolic disorders of a very fundamental nature were induced by that drug. They found marked changes in the ratio of sulphate sulphur to neutral sulphur—the latter form being increased greatly in amount, the former being little affected. They found also that the acidity of the urine increased after chloroform, in some cases remaining high for three days after the anæsthetic was administered. This result was confirmed in 1898 by Thomas (24) who demonstrated a decrease in the alkalinity of the blood during chloroform anæsthesia. Rudenko (25) examined the urines of Salkowski's dog (26) with regard to the sulphur distribution after administration of chloroform by mouth for four consecutive days. An increase of sulphate sulphur appeared only on the last three days; an increase of neutral sulphur on the last two days and on the two days following. There was therefore a marked percentage increase in neutral sulphur on these days, and it was most noticeable on the days following chloroform administration. An attempt to determine whether the neutral sulphur so eliminated was capable of being oxidized by the normal animal failed to give decisive results.

Savelieff (27) confirmed Rudenko's results and those of Salkowski by a single experiment on a dog in whose urine nitrogen and sulphur were estimated before and after chloroform water was given by mouth for four consecutive days. On the first chloroform day,

sulphate sulphur increased, on the second, total nitrogen began to increase; neutral sulphur did not increase until the fourth day of chloroform administration. The increase of sulphate sulphur continued during the first day after the chloroform period, that of total nitrogen during two days after, and that of neutral sulphur during three days after. He considered his figures to indicate that chloroform increased protein destruction but diminished oxidations. Becker (28) and Abram (29) found acetone to be present in the urine after chloroform anæsthesia in the majority of human cases studied, a fact since confirmed by Baldwin (30).

In 1897, Vidal (31) published the results of a very comprehensive study of the effect of chloroform on metabolism. Some of the methods which he used are now open to serious criticism. Urea, for example, was estimated by the hypobromite method after precipitation by basic lead acetate or by phosphotungstic acid; creatinin by the old Neubauer method. The results of a number of observations on human subjects, showed total nitrogen to undergo a marked increase, usually greatest on the second day after anæsthesia. These results were confirmed in experiments on dogs and rabbits. His results on the distribution of nitrogen in the urine and the experiments which he made to determine the causes of variations in the different urinary constituents cannot be regarded as convincing. His results on sulphur elimination confirmed those of Rudenko, Kast and Mester, and Savelieff. The increase in total sulphur elimination was parallel to the excretion of total nitrogen.

The experiments of Doyen and Billet (32) may be mentioned here on account of the relation which they bear to the later work of Paton. By giving two grams of chloroform per kilo dissolved in oil by mouth, they were able to induce in dogs intense necrotic changes in the liver with diminished coagulability of the blood. They considered that chloroform under the conditions of their experiments exercised a selective action upon the liver. Paton's (33) recently published series of experiments was directed specifically toward the elucidation of the question of delayed chloroform poisoning. Dogs in a condition of nitrogenous equilibrium were poisoned with chloroform by inhalation, by mouth, and by subcutaneous injection. When given by inhalation for one, two or three hours, there was a slight increase in urinary nitrogen, accompanied by either no change or a percentage increase in urea nitrogen. He attributes these effects to a stimulation of the urea-forming function in the liver. Changes in ammonia nitrogen (Schlössing's method) were not constant, creatinin excretion was parallel with total nitrogen, and sulphur was not changed. When chloroform (20

grams in 75 cubic centimeters of oil) was given by mouth on two consecutive days, greater changes were produced. The increase in total nitrogen was accompanied by a relative decrease in urea, especially marked on the second chloroform day and the two succeeding days. Ammonia nitrogen was increased both absolutely and relatively. Creatinin increased in absolute figures but suffered a relative decrease. Uric acid and undetermined nitrogen were increased. Sulphate sulphur suffered a relative decrease, neutral sulphur a relative increase. He considers that these experiments show a direct toxic action on the liver, and believes that the greater metabolic toxicity of chloroform given by mouth indicates that if chloroform given by the lungs is not eliminated with normal rapidity, serious injuries might be effected which would lead to symptoms of late chloroform poisoning. He suggests that delayed elimination occurred in his experiments in which chloroform was given by mouth because of unusually firm fixation of chloroform by the proteins of the tissues, this in turn being dependent upon slow absorption of chloroform from the alimentary tract into the blood. He expressed the belief that late chloroform poisoning may arise as the result of chloroform given by inhalation, if elimination of chloroform by the lungs is delayed owing to respiratory deficiency or to unusually firm fixation of chloroform by the tissues. His hypothesis is not convincing because the records fail to show that respiratory deficiency is a constant feature in anæsthesia followed by delayed poisoning in man; no suggestion is offered as to the possible nature of an "unusually firm fixation" of chloroform by the tissue proteins, and the experiments of Moore and Roaf (34) show that this fixation is dependent only upon the vapor pressure of chloroform.

Further chemical evidence regarding late chloroform poisoning is furnished by Taylor (35) and by Wells (36) who analyzed livers, obtained at autopsy, of cases in which death was due to this condition. Taylor was able to isolate leucin, tyrosin and arginin in relatively large amounts; Wells separated histidin, leucin, tyrosin, glycocoll and glutamic acid. In Well's case, a loss of about one-third of the total solids of the liver had occurred and an increase of ether-soluble material was due to infiltrated fat.

On the basis of a consideration of the part played by autolysis in tissue disintegration and of the selective action of chloroform upon metabolic processes, Wells (37) has offered an explanation of the production of tissue changes and of late poisoning by this substance. He emphasized the fact that chloroform is a protoplasmic poison which does not affect the activity of autolytic enzymes but prevents syntheses and oxidations—processes most closely connected with the vitality of cells. As a result of autolysis, tissues subjected to the influence of chloroform may undergo disintegration, the products of their disintegration accumulate and become toxic. Toxic products, in his view, may also accumulate because of impaired function of the liver and kidney. Predisposition to late chloroform poisoning he believes to be connected with influences which decrease oxidations.

The experimental results which we have cited lead to the conclusion that a marked increase of protein catabolism is caused by the action of chloroform upon the tissues which is independent of

the narcotic properties of the substance. The increased catabolism persists for a relatively long time after the administration of chloroform. When chloroform is given by mouth, its effect upon metabolism is more intense than when given by inhalation. It is logical to believe that this fact is due to the greater concentration in which chloroform reaches the liver when given by this path. Other manifestations of the toxic action of chloroform upon metabolism are the increase in uric acid and ammonia, relative decrease in urea, increase in sulphur excretion in which neutral sulphur is chiefly concerned, and decrease in alkalinity of the blood with increased acidity of the urine.

It is a striking fact that in none of the experiments upon which these conclusions are based has late poisoning actually been produced. In the papers in which attention has been directed toward this condition, it is assumed that a mere intensification of the observed effects of chloroform will lead to the condition of late poisoning. Direct evidence concerning the nature of the condition is necessary in testing the truth of this assumption. The observations that necrotic changes in the liver may constantly be found after chloroform anæsthesia in dogs, and that dogs severely poisoned may die from the late effects of the anæsthetic, led us to the hope that by producing the condition experimentally, by studying the morphological changes and the urinary excretions and by comparing these results with those similarly obtained in animals in which recovery from severe poisoning was normal, we could discover in what respects the phenomena of late chloroform poisoning are to be differentiated from those of a normal anæsthesia. We hoped also that such results might point to the nature of the predisposition which is a necessary condition in the production of late poisoning in human subjects. We have succeeded in making such a study in two dogs in which fatal, late poisoning was developed, and in one dog in which severe poisoning, followed by normal recovery, occurred. The description and results of these experiments follow the account of five of the preliminary observations which were the direct impetus to them.

PRELIMINARY OBSERVATIONS.

Dog XVI (A).—Medium sized mongrel bitch. This animal was very resistant to the delayed effect of chloroform.

Mar. 1, '08. Chloroform by inhalation for five hours, twenty-five minutes.

Mar. 2, '08. Chloroform by inhalation for five hours, thirty minutes.

Mar. 3, '08. Chloroform by inhalation for four hours, ten minutes.

Mar. 4, '08. Was perfectly intelligent and bright and only a little weak.

Mar. 5, '08. Seemed well. Killed with chloroform. Autopsy at once. Liver: very yellow and mottled with small red points. Kidneys: in cortex yellowish lines leading down to pelvis. Intestines contained altered blood. Few hemorrhages in ileum.

Micros.—Liver: almost all cells heavily loaded with fat. Central zones of lobules contain a moderate number of necrotic cells that have not shrunken but whose protoplasm stains intensely with eosin and whose nuclei do not stain. No hemorrhage. Kidneys: large amount of fat in the cells of the collecting tubules.

Dog XVI (C).—Small bull dog.

Mar. 13, '08. Given chloroform for five hours. Recovered promptly.

Mar. 14, '08. Seemed well but a little weak. Given chloroform for one and three-fourths hours in A. M., and for one and one-fourth hours in P. M. Came out of anæsthetic slowly.

Mar. 15, '08. Found dead, still warm, and no rigor mortis. Autopsy. Liver: very yellow and studded with numerous dark red areas evidently in center of lobules. Kidneys: apparently unaltered. Intestines: contained altered blood and many small hemorrhages in mucous membrane of ileum. Mesentery: a few small pinhead hemorrhages.

Micros.—Liver: a few islands of cells scattered about the portal spaces are heavily loaded with fat and stain normally with hematoxylin and eosin. The whole center of the lobule is hemorrhagic, the cells are shrunken and stain deep pink and their nuclei are pyknotic.

Dog XVI (D).—Small mongrel fox terrier. This dog was very susceptible to the delayed action of chloroform.

Mar. 16, '08. Chloroform for two hours in A. M. and two hours in P. M., a total of four hours. Came out well from the anæsthetic.

Mar. 17, '08. Very weak and prostrated in A. M.; gradually grew feeble in P. M.; respirations very slow (9 per minute) and had several convulsions. Was moribund and was killed with chloroform. Autopsy at once. Liver: profoundly altered. Very yellow and mottled with dark red areas. Kidneys: apparently unaltered. Intestines: contained bloody feces. Few small hemorrhages in ileum.

Micros.—Liver: a few cells at the periphery of the lobule contain fat and stain in a normal manner. The rest of the cells of the lobule, while not diminished in size, stain deep pink with eosin and their nuclei either do not stain or are pyknotic. There is moderate congestion in the lobules, but no hemorrhage.

Dog XVI (E).—Small black and tan terrier.

Nov. 27, '08. Anæsthetized lightly with chloroform for two hours. Thereafter seemed perfectly well. Killed sixty hours later and autopsy performed

at once. Liver: very yellow. Moderate congestion. Kidneys: normal, except for yellowish lines leading down to the pelvis. Other organs normal.

Micros.—Liver: the cells in the intermediary zone of the lobules are full of fat, those at the periphery contain none. In the central zone the cells are shrunken, the protoplasm forms only a narrow rim around the nuclei, and from many intercapillary spaces the cells have entirely disappeared. A few cells are found staining deep pink with eosin. Kidney: the cells in the collecting tubules contain a large amount of fat.

Dog XVI (F).—Small black and tan mongrel.

Dec. 3, '08. Given chloroform by inhalation for one hour. Not under deeply and came out very promptly.

Dec. 4, '08. To all appearances quite normal.

Dec. 5, '08. Seemed quite normal. Killed with chloroform. Autopsy at once. Liver: very yellow. No other change. Kidneys: normal.

Micros.—Liver: very fatty throughout. Moderate congestion in center of lobules. There is undoubted necrosis of a few cells in the center of each lobule. Their nuclei do not stain and the protoplasm itself takes a deep pink stain with eosin. Kidneys: marked amount of fat in the straight tubules. Other organs normal.

METABOLISM EXPERIMENTS.

Methods.—The animals used were dogs. The urine was tested before the beginning of each experiment and in each of the animals used was found to be acid in reaction and practically free from albumin. On account of the frequency with which vomiting occurs after anæsthesia with chloroform, no food was given during any of the experiments. Osterberg and Wolf (38) and Underhill and Kleiner (39) have shown that the metabolism of fasting dogs is practically constant from a day or two after the beginning of starvation for many days. The first day of each experiment began twenty-four hours after the last feeding, except in the case of Dog I, in which it was over forty-eight hours. Water was given *ad libitum*. The animals were kept in well ventilated cages suited to the quantitative collection of urine. Most of the urine was collected by catheter. Aseptic precautions were observed in the process of catheterization and the bladder was washed daily with four per cent. boric acid solution. In none of the experiments was there evidence of an infection of the bladder. When urine was passed voluntarily in the cage it flowed immediately into a jar containing powdered thymol.

Vomiting occurred on the days following the administration of chloroform. During the first half of each twenty-four hour period contamination of urine with vomitus was always prevented. During the second half this was not always possible. The vomitus, however, was ejected immediately after drinking water and consisted of clear watery fluid, containing a little bile and mucous. The mixture of vomitus and urine after filtration gave no reaction for albumin with heat and acetic acid and we are disposed to think that accidents of this sort were of little significance. When this occurred, the uncontaminated urine of the first half of the day was analyzed separately from the contaminated urine of the second half.

Chloroform (Squibb's, for anæsthesia) was administered by an open cone

and enough was given to produce and maintain surgical anæsthesia, *i. e.*, complete muscular relaxation with absence of the conjunctival reflex. The actual amounts of chloroform used, or the percentage strengths of the chloroform vapor inhaled, were not estimated. During the periods of narcosis, the bodily heat of the animals was maintained by an electric lamp suspended several inches above the body. No fall of temperature occurred in any experiment. The total urine of the twenty-four-hour period, except where otherwise specified, was combined with washings of the bladder and of the collecting tray of the cage and made up to known volume with water. Total nitrogen was estimated by the Kjeldahl method; urea, ammonia, creatinin and creatin by the methods of Folin. The different forms of sulphur were estimated as barium sulphate following the procedures recommended by Folin.

The tissues were fixed in 5 and 10 per cent. formalin for frozen sections and in Müller's fluid and formalin (10 per cent.) equal parts for parafine and celloidin sections. Fat was stained with Scharlach R. The parafin and celloidin sections were stained with hematoxylin and eosin, with Weigert's hematoxylin and with eosin and methylene blue.

DESCRIPTION OF EXPERIMENTS.

Experiment 1.—White, bull bitch pup; weight, 7.65 kilos.

Normal period, June 11 and 12.—Catheterized daily at 9.45 A. M. and bladder washed with boric acid solution.

Chloroform day, June 13.—Catheterized at 9.45 A. M. Chloroform administered from 10.30 A. M. to 12.30 P. M. Urine collected at 12.30 by catheter, 10 c.c. Total urine till 2.30 P. M., 75 c.c. Chloroform again administered from 3.15 to 4.45 P. M. Total period of anæsthesia, three and one-half hours. At end of second period, 35 c.c. of urine were obtained by catheter. Recovery from the second anæsthesia was slow; for over an hour the animal remained in the second stage of anæsthesia and during the evening was very weak. The following morning she appeared normal except for weakness and general depression. No vomiting occurred.

After period, June 14–16.—No noteworthy symptoms occurred. There was no vomiting and feces passed on June 14 were normal in appearance and consistency.

At the end of the experiment, the animal was killed with ether and the autopsy immediately performed.

Autopsy.—Animal much emaciated, adipose tissue practically absent. Heart and lungs normal. Stomach and intestines normal. A small amount of bile-stained fluid was found in the stomach, duodenum and lower end of the ileum. Pancreas and spleen appeared normal. Liver apparently of normal size; surface smooth, consistency about normal; color strikingly yellow; cut sections showed lobulations plainly; centers of lobules appeared grayish and periphery very yellow. No congestion. Gall bladder was full of greenish bile. Kidneys were of normal size and consistency; surface normal. On section, the periphery of the cortex was rather lighter in color than the remainder.

Microscopical Examination. Liver.—This is very fatty. The fat is chiefly, but not entirely, confined to the periphery of the lobules. Stained with hema-

toxylin and eosin, it is seen that around the interlobular spaces in the periphery of the lobules the liver cells retain well their form and staining properties. Within the cells appear numerous larger and smaller round, clear areas from which fat has evidently been dissolved. In the intermediary and central zones great changes have taken place. The liver cells are in all stages of degeneration. Some show only a faintly staining nucleus and the reticular structure of the cell; others stain very badly—both the nucleus and protoplasm. Other cells consist only of a shrunken and distorted and deeply staining nucleus surrounded by a very narrow rim of protoplasm. From some intercapillary areas the cells have entirely disappeared. Confined almost entirely to the intermediary and central zones of the lobules are scattered giant cells containing within themselves masses which stain dark blue with hematoxylin. These masses are soluble in dilute acetic acid without the production of gas bubbles, stain black with a dilute solution of nitrate of silver, and are therefore composed of calcium phosphate. Only in a few instances are they found among the apparently healthy cells. The number of these in each lobule varies between ten and fifty. The capillaries in the central zones contain a few red blood cells. There are no thromboses.

Kidneys.—There is a moderate fatty deposit (not much more than is ordinarily found in dogs) in the cells of the limbs of Henle's loop and in the collecting tubules. The glomeruli are normal. There is a marked degenerative change in the cells of the tubules, especially of the convoluted tubules. The cells are in places swollen so as almost to occlude the lumina of the tubules, their borders are irregular and fragments of them have been broken off. In other tubules nothing but nuclei surrounded by a narrow rim of protoplasm remain, and in still others the cells are retained but the nuclei do not stain. Many tubules contain fragments of cells and casts.

Heart.—The muscle retains its transverse striations.

Gastro-Intestinal Tract.—Normal.

EXPERIMENT 2.—Full-grown fox terrier bitch; weight, 6.78 kilos.

Normal period, June 23 and 24.—Catheterized daily at 9.00 A. M., and bladder washed with boric acid solution.

Chloroform day, June 25.—After catheterization at 9.00 A. M., administration of chloroform was begun at 9.38 A. M., and continued till 12.08 P. M. Twice during this period artificial respiration was necessary. Chloroform was again given from 4.10 to 6.10 P. M. Artificial respiration was necessary twice during this period. Total duration of anæsthesia, four and one-half hours. Recovery from the effects of the anæsthetic was slow but uneventful.

After period, June 26–28.—Catheterized and bladder washed twice daily at twelve-hour intervals. Vomiting occurred after water was taken on June 26. During the day contamination of the urine from this cause was prevented, but the large volume of urine for the twenty-four hours indicates that urine was mixed with vomitus during the night. Except for the presence of a little frothy mucus the urine appeared normal, and after filtration gave no reaction for albumin. No vomiting occurred on June 27 and 28. Feces containing old blood were passed on June 27.

Second chloroform day, June 29.—Chloroform was administered from 10.15 to 11.45 A. M., and again from 1.45 to 3.15 P. M. Total duration of anæsthesia, three hours. The course of the anæsthesia was uneventful but recovery was

slow, the animal being completely prostrated until about 7.00 P. M. Urine secreted from 9.00 A. M. to 7.00 P. M., was collected by catheter and analyzed separately (29 a). On the following morning, at 7.30 A. M., the animal could stand and walk about and, except for weakness and general depression, appeared to be normal in behavior. From 8.30 A. M. till death occurred, at about 10.00 A. M., she was completely prostrated, and, during this time, frequent convulsions occurred. They began with increase in depth and frequency of respiration, twitching and rigidity of the neck muscles; then followed clonic spasms of the legs, and finally a tetanic condition of the whole body. The seizures resembled the convulsions produced by potassium cyanide. The mucous membranes of the mouth, nose and eyes were noticeably bright pink in color. Respiration was markedly increased after each convulsion. In the intervals between the convulsions, prostration was very great, the animal was unconscious but the reflexes were retained. During this time black fluid feces which apparently consisted of old blood were passed. Vomitus of a similar character was ejected. Death occurred at 10 A. M.

The urine which had been passed during the night (7.00 P. M. to 7.00 A. M.) was found mixed with feces or vomitus or both, similar in character to that just mentioned. To this mixture was added the urine collected by catheter at 9.00 A. M., the whole heated to boiling, acetic acid added to complete coagulation, the mixture cooled, made up to 500 c.c., and filtered. The filtrate gave a fairly intense biuret reaction. Aliquot portions of this filtrate were used for the analyses (29 *b*).

Autopsy.—Made immediately after death. The animal was not greatly emaciated, subcutaneous fat being fairly abundant. A curious, vivid, pinkish hue was noticed in the subcutaneous tissues and muscles. Heart and lungs appeared normal. The peritoneum appeared smooth and glistening. Omentum was congested and in it two small areas of hemorrhage were found. It was dotted here and there with typical fat necroses in all stages of formation; none exceeded 4 mm. in diameter. These necroses were found in the fat surrounding the pancreas, within the pancreas itself and in the mesentery of the colon. The pancreas was very firm and pale. There was no sign of obstruction in its ducts. The gall bladder was full of greenish bile. The cystic and common ducts were patent. Liver was of normal size and very yellow. On the surface and on cut section it was dotted with very numerous reddish areas of pinhead size, which were evidently in the center of the lobules. The spleen was normal. Kidneys: cortex slightly yellowish in the neighborhood of the pyramids; otherwise they appeared normal. The mesenteric glands were enlarged and soft. The stomach contained hemorrhagic material, but the mucosa appeared normal. The whole intestinal tract was full of hemorrhagic material. The mucosa of the entire small intestine was hemorrhagic, less marked in the upper third of the ileum than in the duodenum and lower two-thirds of the ileum. The colon was congested near the ileo-cæcal juncture.

Microscopic Examination. Liver.—There is not a normal parenchymatous cell to be found in any of the sections. About the portal spaces are a few small islands of cells that retain their approximate form and staining properties but are very fatty. There are hemorrhagic areas occupying the center of the lobules. Within these areas the liver cells are in all stages of dissolution.

Many are swollen, their nuclei are disintegrated or absent and they stain deep pink with eosin. Others have degenerated to amorphous masses. The remainder of the lobules are composed of swollen cells of which only the nuclei and the reticular structure of the cell may be seen. Scattered among these hydropic cells are others that stain deep pink and are without nuclei. In the hemorrhagic areas only a few small masses stain red with Scharlach R, while in the periphery most of the cells stain deep red. No thromboses are found.

Kidneys.—These contain rather more fat than in Dog 1 but in the same situation. The same changes are to be found in the tubules as in Dog 1 but they are not quite so marked.

Pancreas.—The substance of the pancreas itself is normal. In the fat immediately surrounding the pancreas and in the omentum and mesentery are numerous small hemorrhages and typical fat necroses. The necrotic areas are surrounded by hemorrhage and accumulations of polymorphonuclear leucocytes in great numbers.

Heart.—Striations are present in the heart muscle.

Gastro-Intestinal Tract.—The mucous membrane is congested throughout. In the stomach and large intestine a few hemorrhages are seen; in the small intestine they are almost continuous throughout its whole extent.

Experiment 3.—Young white bull-bitch; weight, 8.52 kilos.

Normal period, July 4 and 5.—Catheterized and bladder washed daily at 9.30 A. M.

Chloroform day, July 6.—Chloroform given from 9.40 A. M. till 12.40 P. M., and from 3.20 till 5.50 P. M. Total duration of anæsthesia five and one-half hours. Urine secreted between 9.30 A. M. and 9.30 P. M. measured 82 c.c., and was analyzed separately (6 *a*). Urine passed during the night (9.30 P. M. to 9.30 A. M.) was contaminated with clear watery vomitus and contained some mucus. This portion was analyzed separately (6 *b*).

After period, July 7 and 8.—During July 7, vomiting occurred very frequently after water was taken. The vomitus did not contain blood. Contamination of the urine was prevented. Feces passes on July 7 were of fairly firm consistency and contained both fresh and old blood. Aside from weakness and these gastro-intestinal symptoms, the animal appeared normal.

July 8.—Until 4.00 P. M. the animal was conscious and apparently normal except for great muscular weakness, and gastro-intestinal symptoms. She stood or walked with great difficulty. Vomiting was frequent; the vomitus contained bloody mucus. At about 4.00 P. M. she became prostrated and nearly unconscious. She lay on her side and at intervals whined in a semi-conscious manner, snarling and biting aimlessly at the floor of the cage. The respiration was very rapid, sometimes deep and labored. The mucous membranes of the mouth, nose and eyes were very noticeably bright pink in color. In the evening at about 9.30 she was prostrated and unconscious, though the reflexes were retained. Respiration, 134 per minute; pulse, 220 per minute; rectal temperature, 41° C. (normal for this animal, 38.8° C.). Her stupor was interrupted by periods of delirious crying, aimless and unconscious or semi-conscious attempts at biting and incoördinated muscular movements. She was catheterized at 9.30 P. M. Death occurred during the night and on the following morning she was found in a state of rigor. The jaws were smeared with bloody vomitus, the hind-

quarters with bloody feces, and the urine receiver contained a mixture of urine with bloody vomitus and feces. The urine collected between 9.30 A. M. and 9.30 P. M. was analyzed. The results obtained, as well as the volume of urine, have been doubled in order to make the figures comparable with those obtained on previous days.

Autopsy.—Made soon after the death of the animal was discovered. Emaciation was not extreme. An unusual vivid pinkish hue was noticed in the subcutaneous tissues and muscles. Hemorrhagic extravasations were found in the tissues of the mediastinum and pleura. The lungs were normal. The pericardium and epicardium contained hemorrhagic areas. The myocardium was pale. The parietal peritoneum contained no hemorrhages. The omentum showed areas of marked congestion and contained a few scattered points of hemorrhage. There were numerous scattered areas of hemorrhage in the mesentery, close to its intestinal attachment. The spleen was somewhat shrunken; no follicles were seen on section. The pancreas was firm and pink in color. The liver was apparently somewhat smaller than normal. Its substance was soft and easily friable. In appearance it resembled that of Dog 2, being very yellow with reddish areas apparently at the center of the lobules. Such areas were larger than in the liver of Dog 2. The gall bladder was filled with dark green bile. The kidneys were of normal size, the tissues somewhat soft and the cortex pale in color. The stomach contained bloody material of fluid consistency; its mucosa was slightly congested. The intestines were filled with blackish fluid material, evidently consisting chiefly of blood. An accurate judgment concerning the condition of the intestinal mucosa was difficult on account of post-mortem change which was considerable. It appeared congested and hemorrhagic, especially in the duodenum and lower third of the ileum. The mesenteric lymph nodes were large and soft.

Microscopic Examination. Liver.—This is greatly altered. At the periphery of the lobules the cells stain well but are very fatty. The inner two-thirds of each lobule are hemorrhagic. In the hemorrhagic areas the cells are swollen, stain deeply with eosin, and are without nuclei. Giant cells containing calcium phosphate are present in the hemorrhagic areas, but are confined to their periphery and are not found in the center. No thromboses are to be seen.

Kidneys.—These are greatly congested. There are small hemorrhages in the cortex as well as the pyramids, and one or two within the glomeruli themselves. In other respects the changes are like those that have been described.

The results which have been obtained are included in the tables on pages 367, 368, 369 and 370. Some of the results have been plotted in the form of curves and may be found on pages 359, 361 and 362. Other data not included in the tables are as follows: a faint reaction for albumin was obtained in the urine of each dog on the days on which the chloroform was given. The urine showed a slight reducing power when tested with Fehling's solution on these days. The urine of each dog on the days on which chloroform was administered was highly colored and gave strong reac-

tions for bile pigment. This reaction persisted until the end of the experiment and in the cases of Dogs 2 and 3 was very intense. Gerhardt's test for diacetic acid and Legal's test for acetone was negative in every urine. Every urine was acid to litmus.

The limits of this paper do not include a consideration of the symptoms exhibited by human cases, but a word in regard to the similarity to them of those observed in our dogs may be allowed. In the so-called "cholæmic" or adult type of delayed poisoning, a longer or shorter uneventful period is the rule. And so it was with both our dogs. With Dog 3 at least 28 hours intervened after the anæsthetic before untoward symptoms appeared, and during this time it was impossible to detect any difference from the recovery from any severe narcosis. Human cases become wildly delirious, occasionally have convulsions and coma ends the scene. The signs of mental disorder or the equivalent of mental disorder in a dog were seen in both of ours. In Dog 2 there were convulsions and in Dog 3 purposeless attempts to bite and snarling and howling. Coma supervened in both instances. The vomiting of blood and the bloody stools and the large amount of bile pigment in the urine are common to both animals and human beings.

SUMMARY OF RESULTS.

Total Nitrogen.—In every experiment a greatly increased nitrogen elimination occurred on the day on which chloroform was given and on the following days. On the day of poisoning, it amounted to 48 per cent. in Experiment 1; to 65 per cent. in Experiment 2; and to 108 per cent. in Experiment 3. On the next day after, in every experiment, a still greater increase occurred: 54 per cent. in Experiment 1; 76 per cent. in Experiment 2; and 185 per cent. in Experiment 3. In the two following days, in Experiment 1, a gradual decrease to normal took place. In Experiments 2 and 3, the nitrogen elimination remained high until the end.

Urea and Ammonia.—The curve of excretion of nitrogen in the form of urea and ammonia follows the curve of total nitrogen excretion very closely in Experiments 1 and 3. In Experiment 2 the sum of these forms of nitrogen diminished on the day following chloroform while the total nitrogen increased. A gradual increase then occurred during two days, but on the second chloroform day the increase in total nitrogen was again accompanied by a decrease in urea and ammonia. In every experiment the *percentage* of nitrogen in the form of urea and ammonia decreased on the day of chloroform. This percentage diminution became greater on the next following day in Experiments 1 and 2 and remained the same in Experiment 3. In every experiment it then rose.

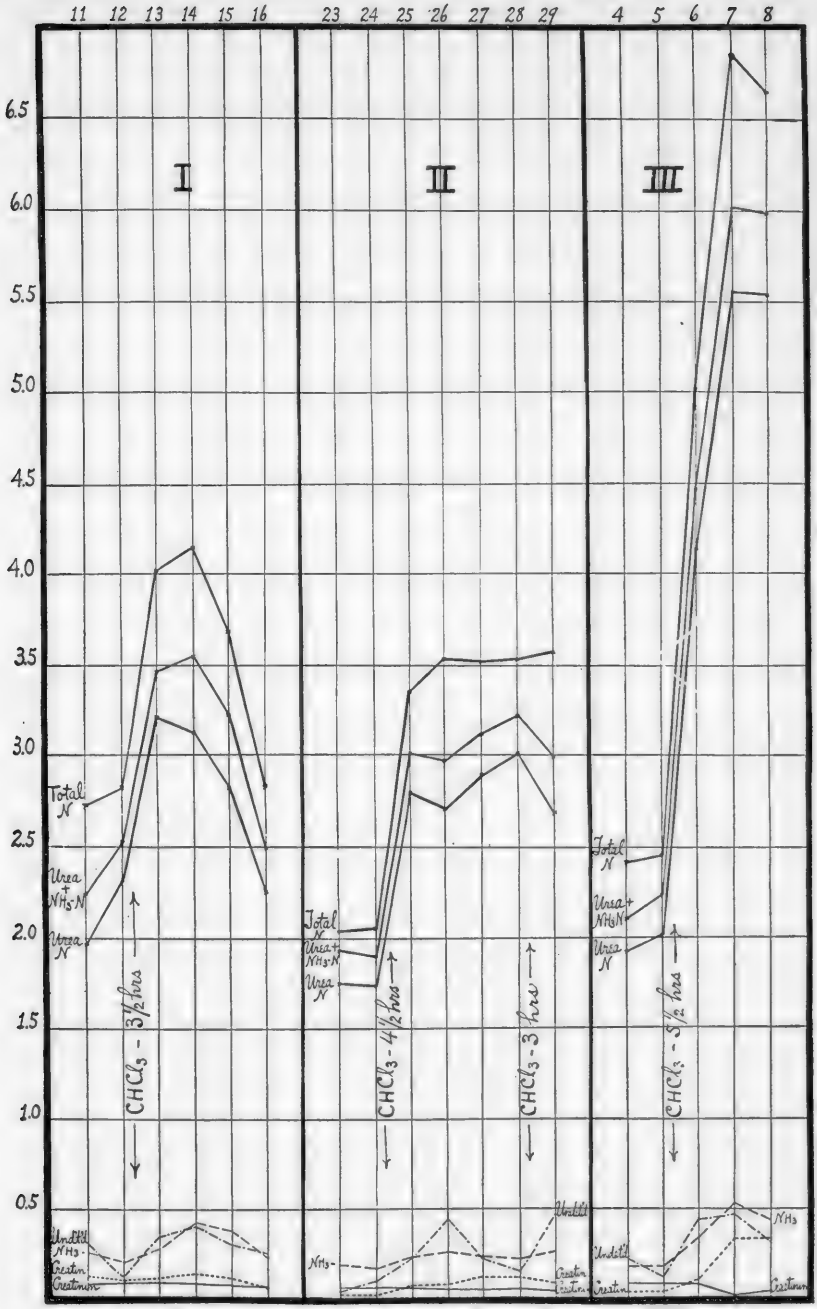

DIAGRAM 1. Excretion of nitrogen in the three metabolism dogs. Ordinates = grams of nitrogen; abscissas = days of experiments.

returning to normal in Experiments 1 and 2. In Experiment 2 the second administration of chloroform again caused a decrease in the percentage of nitrogen as urea and ammonia.

In each experiment on the day of poisoning, the curve of urea nitrogen excretion runs almost exactly parallel with those of total nitrogen and urea and ammonia nitrogen. On the day following poisoning there is a divergence of the urea curve from that of total nitrogen, the percentage of urea nitrogen being further decreased. Although an increase in ammonia nitrogen occurred on each day on which chloroform was given, it was not proportional to the increase in total nitrogen, and hence in every case a percentage decrease was found. A greater increase in ammonia nitrogen occurred in every experiment on the day following chloroform, so that in Experiment 2 the percentage returned to normal, and in Experiments 1 and 3 it exceeded normal. In Experiment 1 and in Experiment 2 till the second chloroform day, a gradual decrease then took place, the percentage figures remaining high only in Experiment 1.

Creatin and Creatinin.—In Experiments 1 and 2 the absolute values for creatinin were constant throughout; the percentage figures are, therefore, decreased on the chloroform days and those following. In Experiment 1 creatin nitrogen increased on the chloroform day and the day following, so that a fairly constant percentage of this form of nitrogen was maintained. In Experiment 2 a greater absolute increase in creatin occurred, so that the percentage was higher than normal on and after the chloroform day. The second administration of chloroform in Experiment 2 did not cause as great an increase in creatin as did the first.

In Experiment 3, creatinin was constant on the two normal days and on the chloroform day. It disappeared altogether on the day following anæsthesia, but reappeared in small amount on the final day. These changes produced a marked decrease in the percentage values. Creatin, constant on the two normal days, rose slightly on the day of anæsthesia, and on the two following days was increased by 200 per cent. This caused a very marked rise in the percentage values.

Undetermined Nitrogen.—The undetermined nitrogen in each experiment increased on the day of anæsthesia. The percentage on this day is high in all and much above normal in Experiment 2. In every experiment a still greater absolute increase took place on the day following chloroform. On this day, in Experiments 1 and 2, there was a percentage increase. In Experiment 3 a decrease in the percentage value occurred. An actual and a percentage fall in undetermined nitrogen occurred on the days of recovery in Experiments 1 and 2, and also on the day of death in Dog 3. In Dog 2, on the second day of poisoning, an actual and percentage increase in undetermined nitrogen took place.

Total Sulphur.—As in the case of total nitrogen, total sulphur was greatly increased on the days of chloroform poisoning in every experiment. This increase amounted to 114 per cent. in Experiment 1; 133 per cent. in Experiment 2; and 170 per cent. in Experiment 3. In Experiment 1, this rise was succeeded by a gradual fall to normal. In Experiments 2 and 3 the total sulphur underwent still greater increase on the day following poisoning; thereafter there was a decrease, but never to normal figures. In none of the experi-

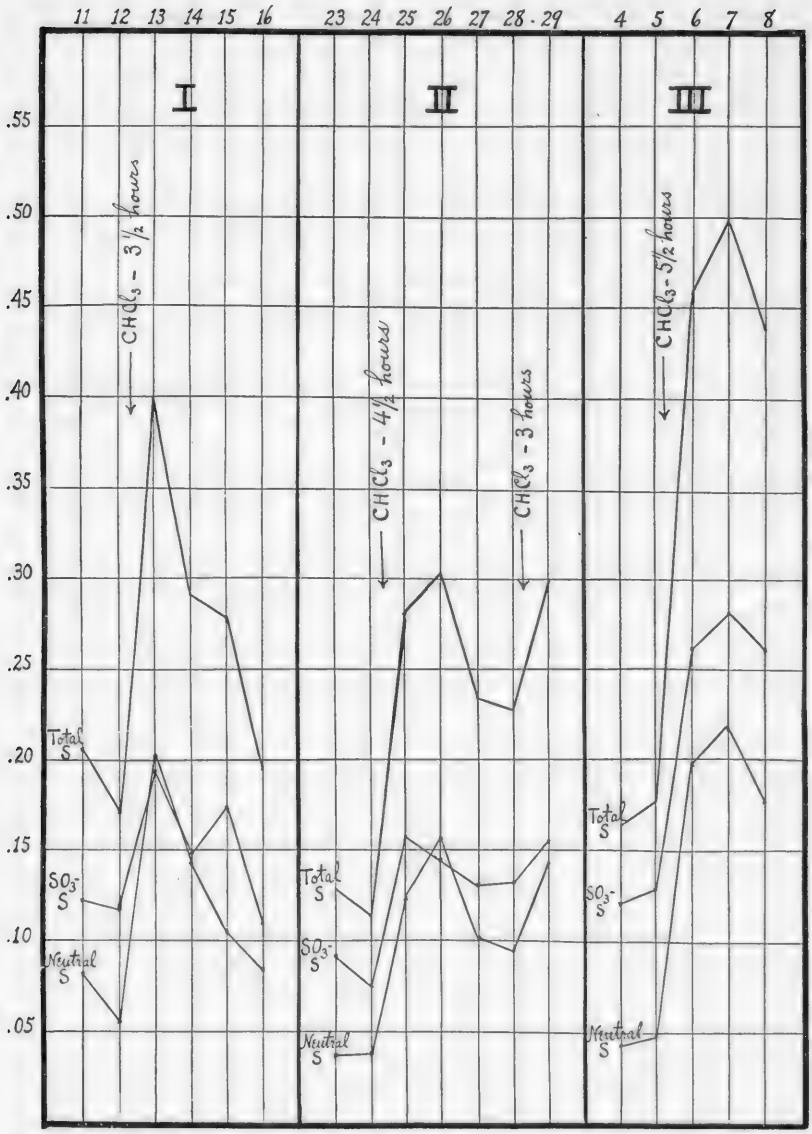

DIAGRAM 2. Excretion of sulphur in the three metabolism dogs. Ordinates = grams of sulphur.

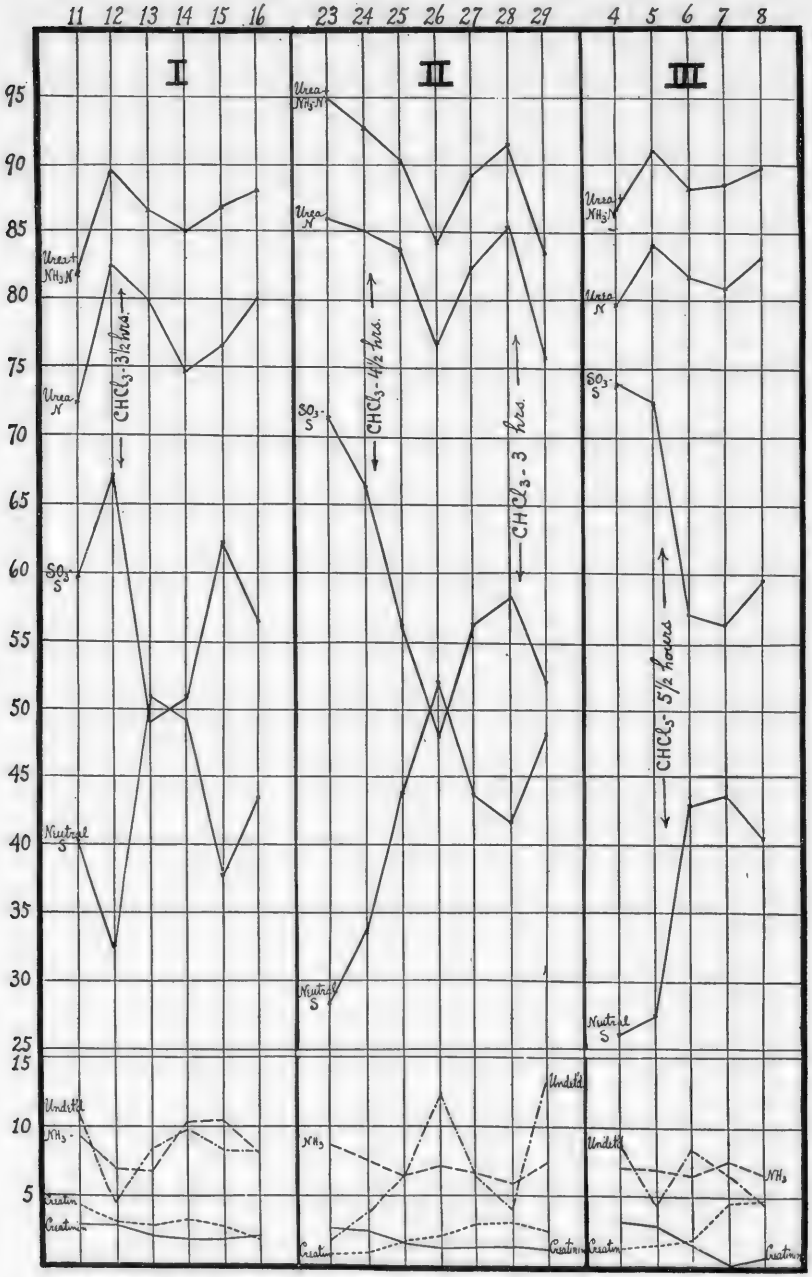

DIAGRAM 3. Distribution of nitrogen and sulphur in the three metabolism dogs. Ordinates = percentages of total nitrogen and total sulphur.

ments was there a close parallelism between total sulphur and total nitrogen elimination.

Sulphate Sulphur and Neutral Sulphur.—On the day of chloroform poisoning in each experiment the increase in neutral sulphur was greater than the increase in sulphate sulphur. On this day, therefore, a marked decrease in the percentage of sulphate sulphur and a corresponding increase in the percentage of neutral sulphur was found. On the day following chloroform in Experiment 1 recovery of the normal sulphur relations began. In Experiment 2, a decrease in sulphate sulphur occurred while neutral sulphur underwent a still further increase. In Experiment 3 on this day both increased. In both experiments (2 and 3), the decrease in percentage of sulphate sulphur and the increase in percentage of neutral sulphur which began on the day of anæsthesia were intensified on the following day. On the second and third days after chloroform in Experiment 2, which were days of recovery, the sulphur values tended to regain normal, but on neither day was the return to normal nearly complete. This is also true of the final day (day of death) in the case of Dog 3. The second day of chloroform in Dog 2 is marked by changes in the sulphur excretion similar to those on the first chloroform day.

DISCUSSION OF CHEMICAL RESULTS.

In the first of the three metabolism experiments which have been described, severe poisoning by chloroform followed by normal recovery has been induced. This experiment may therefore serve as a control for the second and third experiments in which fatal delayed poisoning resulted. In all three experiments the excretion of total nitrogen is greatly increased on the day of poisoning. In Experiment 3 this increase was fifty per cent. greater than in Experiments 1 and 2. In all three the distribution of the nitrogen as urea and ammonia is normal: the absolute values for creatinin are strictly normal. Percentage figures for undetermined nitrogen in Experiments 1 and 3 are within normal limits. The quantitative change in total sulphur excretion varies among the three, being greatest in Experiment 3, but the change in distribution between sulphate sulphur and neutral sulphur is alike in all. It is obvious, then, that on the day of anæsthesia no distinction can be made between the animal which recovered and those in which fatal poisoning ensued on the basis of percentage differences in the distribution of nitrogen as urea, ammonia, creatinin, and undetermined, or in the sulphur distribution. Only in the case of creatin nitrogen is there a difference between the control (1) and the other two dogs. In the control on the day of poisoning it is normal, in the other two a

marked increase occurs. The change in amount of this substance is not proportional to the severity of poisoning, for it was greater in Experiment 2 than in Experiment 3. So far, then, as a comparison of the data for the day of poisoning is concerned, we may conclude that there is nothing in the analytical differences to show which animal will succumb to late poisoning, although a rough index of the severity of the acute effects is given by the total nitrogen and sulphur figures and the changes in creatin.

A similar examination of the figures for the days following the poisoning shows no difference in distribution of nitrogen as urea and ammonia between control Dog 1 and fatally poisoned Dog 3. In both, the percentage figures are normal throughout. Considered separately an apparent difference exists in that the percentage of ammonia nitrogen rises in Experiment 1 and remains nearly constant in Experiment 3; it is also fairly constant in Experiment 2. It is difficult to say whether any importance should be attached to this result or not. The percentage of undetermined nitrogen is within normal limits throughout in Dogs 1 and 3.

Creatinin, which in the control animal was constant up to the second day after poisoning and then suffered only a slight decrease, in Dog 2 was somewhat decreased after the poisoning and markedly decreased after the second poisoning (29 *b*). In Dog 3, it disappeared entirely on the day after poisoning, but reappeared in the half-day preceding death. Creatin, which in the control (1) was normal on the chloroform day, on the day after was moderately increased, and during recovery sank to below the initial figures. In the dogs suffering the severer poisoning, creatin, increased on the days of poisoning, is very strikingly increased on the days after. So far as the nitrogen distribution is concerned, then, the fatally poisoned dogs vary from the control mainly in respect to changes in creatin and creatinin. Recovery of the normal ratio between the sulphate sulphur and neutral sulphur is delayed for a day after poisoning in the control dog; in neither of the other two is the normal relation reached after the poisoning. In this respect, the animals suffering late poisoning differ from the one in which recovery is normal.

These results show that quantitative differences only exist be-

tween the fatally poisoned dogs and the control, and that aside from variations in the amounts of nitrogen and sulphur excreted, the greatest differences are to be found in creatin, creatinin and neutral sulphur.

Definite conclusions as to the significance of these changes are difficult. It is most reasonable to assume that the intense destruction of body protein which the excreted nitrogen and sulphur represent is the result of autolysis. The known property of chloroform of interfering with synthetic and oxidative processes without inhibiting autolytic processes (as pointed out by Wells (40)), together with the great similarity between the late fatal chloroform poisoning and phosphorus poisoning in which increased autolysis has been demonstrated, bear out this view. It is a striking fact and worthy of especial emphasis that the distribution of nitrogen as urea, ammonia and undetermined, so closely approximated the normal. There could have been no excessive elimination of amino-acids, and it is obvious that death could have not been due to accumulation of these bodies, or to an acid intoxication.

In view of our present ignorance concerning the physiological significance of creatin, creatinin and neutral sulphur, attempt to fix the responsibility for the fatal effects of chloroform upon changes in processes having to do with the elaboration of these substances, would be hazardous. Folin is the author of the conception of the intimate relationship between creatinin excretion and endogenous metabolism. Our figures show—as has already been shown in the case of potassium cyanide—that an intense increase of endogenous catabolism may be consistent with markedly diminished creatinin excretion. They might be considered as supporting in a measure the view of Mellanby (41) that the liver is the organ chiefly concerned in the formation of this substance. It is also impossible for us to decide at present whether the increase in neutral sulphur is the result of decreased oxidation of sulphurized substances which under more nearly normal conditions would have been excreted as sulphuric acid or whether its excretion in increased amount represents an increase in processes of metabolism, the specific end products of which include sulphur in this form.

We believe we are justified in the belief that the death of the

two fatally poisoned animals was not due to acid intoxication or to the accumulation of products (amino-acids) of cell digestion. We believe also that death was not due solely to the excessive amount of protein lost, *i. e.*, tissue disintegrated. Under the influence of starvation alone an animal could lose the amount of protein equivalent to the amounts of nitrogen excreted by our dogs without serious consequences.

We are driven to the quite indefinite and unsatisfactory view that death is due to the presence of toxic substances of an unknown nature, which owe their presence either to excessive formation by abnormal metabolic processes, or to the failure on the part of the organism to neutralize in the normal manner toxic substances normally formed.

DISCUSSION OF PATHOLOGICAL RESULTS.

From the experiments of others and those that we have ourselves performed, it is plain that a single anæsthesia with chloroform of a duration of more than half an hour induces fatty changes that are demonstrable in the liver in from twelve to twenty-four hours, reach their maximum in about forty-eight hours and then gradually retrograde, but the excess of fat may be demonstrated for about two weeks. The fat appears first in the intermediary zone and eventually may spread to the periphery and the whole lobule be intensely fatty. With more prolonged, and especially with repeated anæsthesias which are very much more destructive than single ones, necrosis appears. But, as is shown by two of our experiments, anæsthesia of only one or two hours is sufficient, at least at times, to cause necrosis. The necrosis also begins in the center and extends centrifugally, so that almost all the cells may be necrotic and only a few around the portal spaces be still living and these are full of fat. The amount of protoplasm in the necrotic cells varies according to the length of time intervening before death. When this occurs promptly the cells may remain of almost normal size and contour. When longer delayed, the protoplasm diminishes markedly until there may be only the narrowest margin around degenerated nuclei that lie in the intercapillary spaces. The amount of hemorrhagic extravasation varies from none at all to a very con-

siderable amount. Some of our specimens show a degree of this greater than we have seen reported.

TABLE I.

Excretion of Nitrogen in Experiment 1.

Date. June, 1908.	Body Weight.	Volume of Urine.	Total N.	Urea N.	NH_3 N.	Urea + NH_3 N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.	Urea N.	NH_3 N.	Urea + NH_3 N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.
	kilos	c.c.	gms.	gms.	gm.	gms.	gm.	gm.	gm.	gm.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
11	7.18	315	2.7170	1.9710	0.2520	2.2230	0.0809	0.1131	0.1940	0.3000	72.54	9.28	81.82	2.98	4.16	7.14	11.04
12	7.00	240	2.8210	2.3270	0.1950	2.5220	0.0791	0.0914	0.1705	0.1285	82.49	6.91	89.40	2.80	3.24	6.04	4.56
13	6.78	400	4.0170	3.2136	0.2704	3.4840	0.0816	0.1150	0.1966	0.3364	80.00	6.73	86.73	2.03	2.86	4.89	8.37
14	6.68	315	4.1730	3.1226	0.4264	3.5490	0.0772	0.1378	0.2150	0.4090	74.82	10.22	85.04	1.85	3.30	5.15	9.80
15	6.48	705	3.6920	2.8262	0.3848	3.2110	0.0674	0.1052	0.1726	0.3084	76.55	10.42	86.97	1.83	2.85	4.68	8.35
16	6.32	495	2.8210	2.2568	0.2262	2.4830	0.0582	0.0507	0.1089	0.2291	80.00	8.02	88.02	2.06	1.80	3.86	8.12

Very striking in two of our metabolism dogs were the calcium deposits and the giant cells surrounding them. These deposits were always in the necrotic areas and chiefly in the periphery of these. Their presence in less than forty-eight hours after poisoning indicates the rapidity of calcium deposition and engulfing cell formation. There is no mention of such deposits in pathological or experimental literature relating to chloroform. That they follow as a result of the chloroform is shown by the fact that we produced them in two instances.

TABLE II.

Excretion of Sulphur in Experiment 1.

Date. June, 1908.	Total S.	Total SO_3 S.	Inorganic SO_3 S.	Ethereal SO_3 S.	Neutral S.	Total SO_3 S.	Neutral S.
	gm.	gm.	gm.	gm.	gm.	per cent.	per cent.
11	0.2055	0.1228	0.1033	0.0195	0.0827	59.76	40.24
12	0.1739	0.1170	0.1030	0.0140	0.0569	67.28	32.72
13	0.3961	0.1942	0.1783	0.0159	0.2019	49.03	50.97
14	0.2920	0.1486	0.1475	0.0011	0.1434	50.89	49.11
15	0.2799	0.1739	0.1588	0.0151	0.1060	62.13	37.87
16	0.1956	0.1104	0.0926	0.0178	0.0852	56.44	43.56

In our second metabolism experiment mention was made of fat necrosis of the omentum and mesentery. There is no similar record in the literature. That fat necrosis sometimes occurs with hepatic poisons is shown by the reported observation of Wells (42) in regard to hydrazine poisoning. He produced in one dog small and scattered but typical fat necroses. Our result with chloroform stands as an isolated observation.

TABLE III.

Excretion of Nitrogen in Experiment 2.

Date. June, 1908.	Body Weight.	Volume of Urine.	Total N.	Urea N.	NH_3 N.	Urea + NH_3 N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.	Urea N.	NH_3 N.	Urea + NH_3 N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.
	kilos	c.c.	gms.	gm.	gm.	gms.	gm.	gm.	gm.	gm.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
23	6.78	38	2.0280	1.7446	0.1794	1.9240	0.0530	0.0146	0.0676	0.0364	86.02	8.85	94.87	2.61	0.72	3.33	1.80
24	6.62	37	2.0475	1.7420	0.1560	1.8980	0.0511	0.0173	0.0684	0.0811	85.08	7.62	92.70	2.50	0.84	3.34	3.96
25	6.58	240	3.3540	2.8080	0.2210	3.0290	0.0507	0.0543	0.1050	0.2200	83.72	6.58	90.30	1.51	1.62	3.13	6.56
26	6.51	605	3.5360	2.7200	0.2570	2.9770	0.0455	0.0722	0.1177	0.4413	76.92	7.27	84.19	1.29	2.04	3.33	12.48
27	6.48	88	3.5100	2.8938	0.2392	3.1330	0.0462	0.1062	0.1524	0.2246	82.44	6.81	89.25	1.32	3.03	4.35	6.40
28	6.28	165	3.5230	3.0134	0.2106	3.2240	0.0486	0.1084	0.1570	0.1420	85.53	5.98	91.51	1.38	3.08	4.46	4.03
29*a*		110	2.0540	1.6562	0.1638	1.8200	0.0223	0.0648	0.0871	0.1469	80.63	7.98	88.61	1.09	3.15	4.24	7.15
29*b*			1.49175	1.0335	0.1040	1.1375	0.0147	0.0207	0.0354	0.31885	69.28	6.97	76.25	0.98	1.39	2.37	21.38
29[2]	6.10		3.54575	2.6897	0.2678	2.9575	0.0370	0.0855	0.1225	0.46575	75.86	7.55	83.41	1.04	2.41	3.45	13.14

The changes produced in the kidneys and heart have been found by other investigators and by ourselves to be much less constant and severe than in the liver. There is often considerable fat in the kidneys, especially in the cells of the straight and collecting tubules. The presence of fat in this situation in normal dogs renders a conclusion somewhat difficult. Hemorrhages are sometimes found in the kidney. The chief change is in the cells of the convoluted tubules which are at times swollen, at others eroded and often a few necrotic. The lumina of the tubules contain fragments of cells and casts. It is the rule to find the glomeruli entirely unaffected.

The muscles of the heart may show loss of transverse striation

and many fat drops throughout the fibers. Changes in the nuclei have also been described. Frequently, however, no abnormality can be found. From our experience it seems that there is not enough damage done this organ to explain any interference with its functional activity.

TABLE IV.

Excretion of Sulphur in Experiment 2.

Date. June, 1908.	Total S.	Total SO_3 S	Inorganic SO_3 S	Ethereal SO_3 S	Neutral S.	Total SO_3 S.	Neutral S.
	gm.	gm.	gm.	gm.	gm.	per cent.	per cent.
23	0.1299	0.0930	0.0860	0.0070	0.0369	71.57	28.43
24	0.1155	0.0766	0.0718	0.0048	0.0389	66.32	33.68
25	0.2814	0.1579	0.1527	0.0052	0.1235	56.11	43.89
26	0.3035	0.1456	0.1390	0 0066	0.1579	47.98	52.02
27	0.2347	0.1321	0.1264	0.0057	0.1026	56.28	43.72
28	0.2292	0.1335	0.1255	0.0080	0.0957	58.25	41.75
29*a*	0.2015	0.1137	0.1042	0 0095	0.0878	56.43	43.57
29*b*	0.0987	0.0423	0.0368	0.0055	0.0564	42.86	57.14
29[2]	0.3002	0.1560	0.1410	0.0150	0.1442	51.94	48.06

We have found congestion and small areas of hemorrhage in the gastro-intestinal tract frequently, and in one dog the whole small intestine was hemorrhagic. In this same dog were hemorrhages into the peritoneum, pleuræ, pericardium and mediastinal tissue. We have also found hemorrhages in the serous membranes of other dogs. The lesions found elsewhere are negligible.

It may thus be seen that there is a very great similarity between the experimental lesions and those reported in fatal human cases. In both they are most severe in the liver and consist of central necrosis and fatty degeneration and, allowing for a certain difference in structure of the organ, are strictly comparable. The same may be said of the kidney lesions. In both there is also a tendency to hemorrhage into the serous membranes and into the gastro-intestinal tract.

[2] The figures for June 29 are the sum of those obtained in the separate analyses of 29*a* and 29*b*. 29*a* represents the twelve hours during part of which chloroform was administered and the urine of which was uncontaminated. 29*b* represents the following twelve hours, the urine of which was contaminated (see page 352, *i. e.*, description of technique of experiments).

TABLE V.

Excretion of Nitrogen in Experiment 3.

Date. July, 1908.	Body Weight.	Volume of Urine.	Total N.	Urea N.	NH_3 N.	Urea + NH_3 N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.	Urea N.	NH_3 N.	Urea + NH_3 N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.
	kilos	c.c.	gms.	gms.	gm.	gms.	gm.	gm.	gm.	gm	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
4	8.52	100	2.4245	1.9344	0.1716	2.1060	0.0760	0.0274	0.1034	0.2151	79.79	7.08	86.87	3.13	1.13	4.26	8.87
5	8.32	102	2.4570	2.0644	0.1716	2.2360	0.0719	0.0382	0.1101	0.1109	84.02	6.98	91.00	2.93	1.56	4.49	4.51
6*a*		82	2.0475	1.7576	0.1014	1.8590	0.0317	0.0434	0.0751	0.1134	85.84	4.95	90.79	1.55	2.12	3.67	5.54
6*b*			3.0225	2.3868	0.2262	2.6130	0.0404	0.0537	0.0941	0.3154	78.97	7.48	86.45	1.34	1.78	3.12	10.43
6[3]	8.75	82	5.0700	4.1444	0.3276	4.4720	0.0721	0.0971	0.1692	0.4288	81.74	6.46	88.20	1.42	1.92	3.34	8.46
7	7.80	255	6.8705	5.5614	0.5226	6.0840	0.0000	0.3211	0.3211	0.4654	80.95	7.61	88.56	0.00	4.67	4.67	6.77
8[4]	7.42	204	6.6560	5.5432	0.4368	5.9800	0.0482	0.3224	0.3706	0.3054	83.28	6.56	89.84	0.72	4.85	5.57	4.59

We have previously said that from our metabolism studies we were led to believe that the differences between the changes produced by prolonged anæsthesia and those by delayed poisoning were of degree and not of kind. This view is strongly substantiated by

TABLE VI.

Excretion of Sulphur in Experiment 3.

Date. July, 1908.	Total S.	Total SO_3 S.	Neutral S.	Total SO_3 S.	Neutral S.
	gm.	gm.	gm.	per cent.	per cent.
4	0.1647	0.1218	0.0429	73.95	26.05
5	0.1781	0.1292	0.0479	72.54	27.46
6*a*	0.2203	0.1395	0.0808	63.33	36.67
6*b*	0.23965	0.1232	0.11645	51.41	48.59
6[3]	0.4600	0.2627	0.1973	57.11	42.89
7	0.5003	0.2821	0.2182	56.39	43.61
8[4]	0.4398	0.2615	0.1783	59.46	40.54

[3] The figures for July 6 are the sum of those obtained in the separate analyses of 6*a* and 6*b*. 6*a* represents the twelve hours during part of which chloroform was administered and the urine of which was uncontaminated. 6*b* represents the following twelve hours, the urine of which was contaminated by vomitus and feces (see page 352, *i. e.*, description of technique of experiments).

[4] Death occurred during the second half of this day. Urine of the first twelve hours was collected without contamination and the results obtained have been multiplied by two to enable a comparison with those of other days.

the pathological examination. Dog 1 that was recovering and that served as a control had changes exactly similar to those in Dog 3, but they were not so severe. That in this Dog 1 with the extensive liver lesions the metabolism should have been normal at the close, is very remarkable and serves to show that metabolism may not be disturbed even by severe organic lesions in the liver. Furthermore, the many experiments that we have made show unmistakably that with severe liver lesions there may be apparent perfect health, but they also show that any prolonged anæsthesia with chloroform produces in the dog great alteration and even destruction in liver tissue. We believe that this is true of human beings as well as of animals.

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EXPLANATION OF PLATES.

Plates VIII–X.

Fig. 1.[5] Liver of Dog XVI *D*. Shows almost complete necrosis. A few islands of living cells remain.

Fig. 2. Liver of metabolism Dog (2). Stained with Scharlach R. Shows periphery of lobules full of fat. The central necrotic portions do not take the fat stain.

Fig. 3. Liver of metabolism Dog (1). Low power. Shows central necrosis with crystals of calcium phosphate.

Fig. 4. Same as Fig. 3. High power. Shows giant cells engulfing calcium crystals.

Fig. 5. Liver of metabolism Dog (3). Low power. Note similarity between this and Fig. 3. Shows also calcium phosphate crystals but more extensive necrosis.

[5] For all these photographs we are indebted to Dr. Edward Leaming of the Rockefeller Institute.

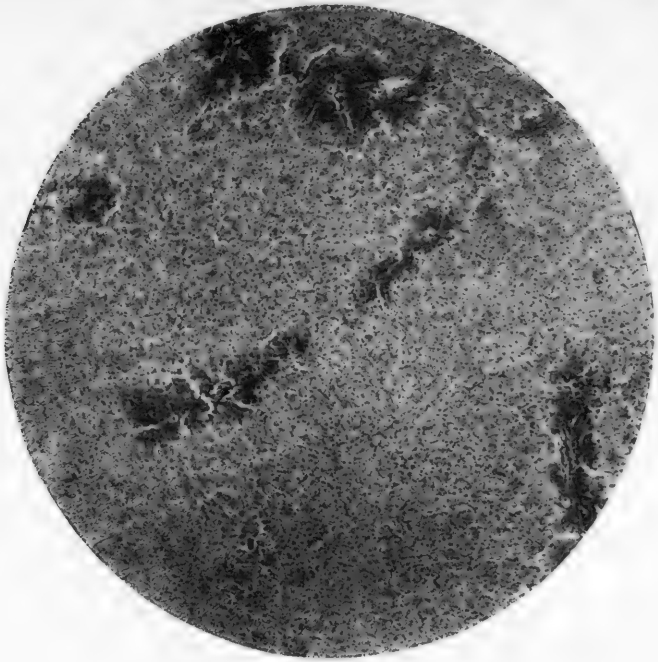

FIG. 1.

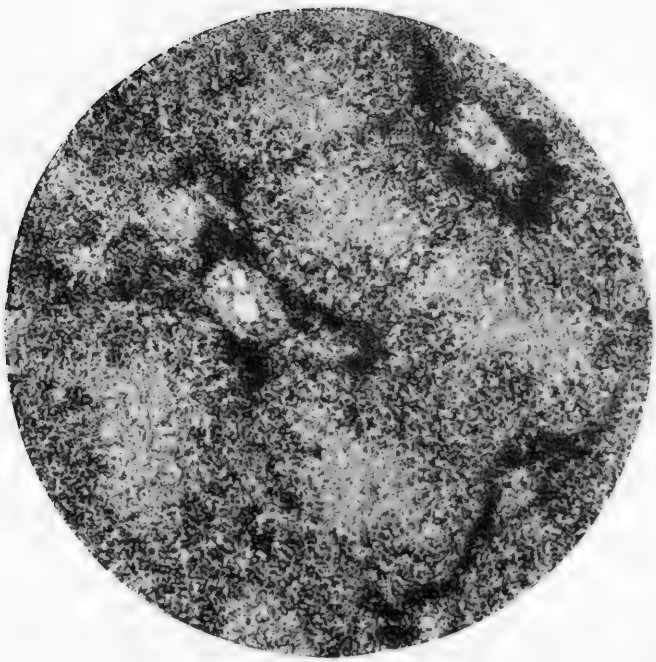

FIG. 2.

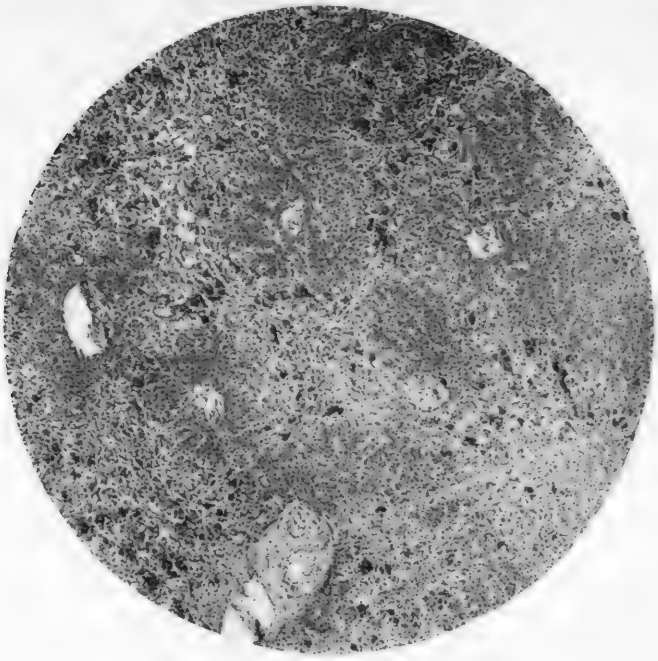

FIG. 3.

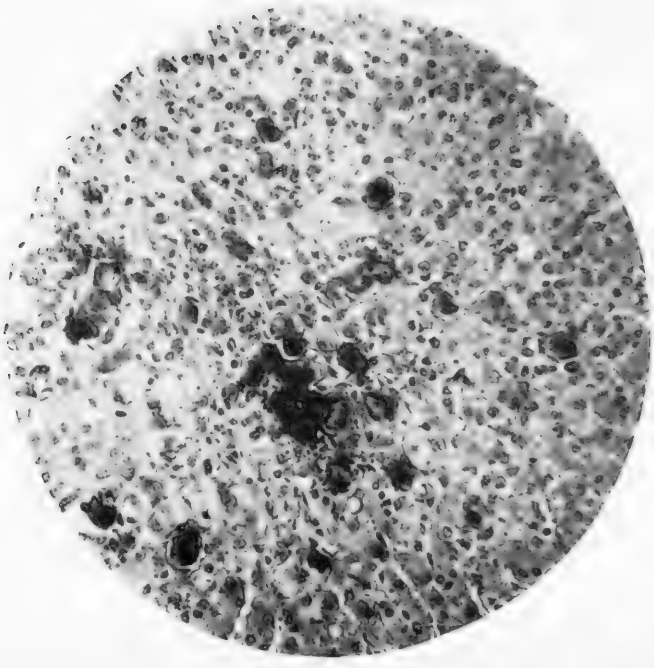

FIG. 4.

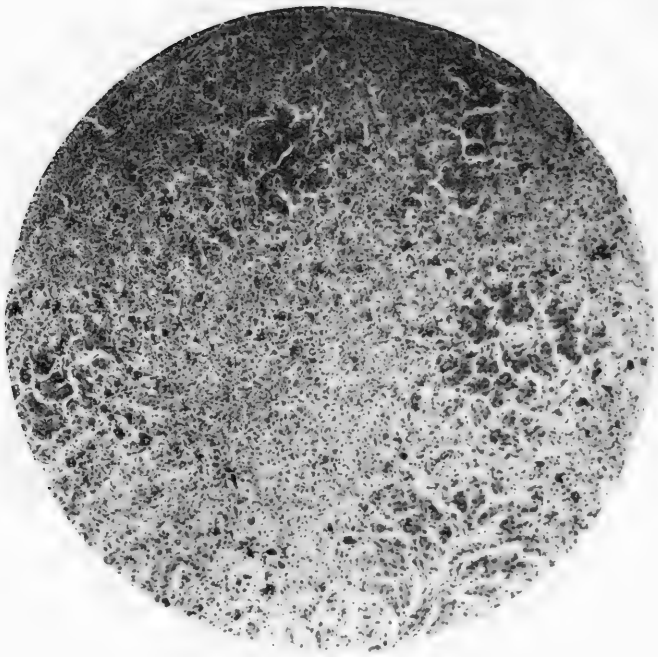

FIG. 5.

PARTIAL, PROGRESSIVE AND COMPLETE OCCLUSION OF THE AORTA AND OTHER LARGE ARTERIES IN THE DOG BY MEANS OF THE METAL BAND.[1]

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PLATES XI–XIII.

At the meeting of the Johns Hopkins Hospital Medical Society, March 20, 1905, a brief preliminary report was made of the results of experiments in occlusion upon the abdominal aorta and other arteries of sixty-three dogs, conducted during the previous winter and autumn by Dr. W. F. M. Sowers and me.[2] During the following year these investigations were continued with the assistance of Dr. E. H. Richardson, thirty-nine dogs being operated upon. With the aid of a modified Brauer apparatus devised by Dr. Follis and Dr. Fisher we were able successfully to constrict the thoracic aorta both in animals and man. In June, 1906, at the meeting of the American Medical Association in Boston, attention was again called to our work, the results to date being given in merest outline.[3] During the following two years, the exigencies of the surgical clinic seemed to demand experimental investigation in another direction and hence only a little time was found for prosecuting the arterial work under consideration, although several problems which have arisen in connection with it we regard with unabated interest; and with the solution of these we are again concerning ourselves. In the meantime, however, a number of experiments have been made, and several animals which were for many months under observation have furnished additional facts worthy of record. The method of applying the band has been improved and modified and new instruments for rolling it devised. The full report of this work will probably be

[1] Received for publication, January 14, 1909.

[2] *Johns Hopkins Hospital Bulletin*, 1905, xvi, 346.

[3] *Jour. of the Amer. Med. Assoc.*, 1906, xlvii, 2147.

published during the year in the Johns Hopkins Hospital Reports.

The incentive to the work was the desire, experienced by so many surgeons of the past and present, to be able to occlude safely the abdominal aorta in the hope of curing thereby aneurisms of this vessel and of the common iliac arteries. I shall write later of the attempts of Dubois, Assalini, Bujalsky, Pirogoff, Luigi Porta, Cooper and Keen to compress gradually the abdominal aorta by means of specially devised instruments which, passed through an incision in the abdominal wall, carried a snare of silk, catgut or metal which might at any moment be tightened or loosened at will. A fault common to all of the methods hitherto devised is seemingly an insurmountable one—the difficulty of preventing sepsis in the track of an instrument maintaining direct communication for days or even weeks with the air. A better method might, we thought, be one permitting, in each entre-act, complete closure of the wound. The material compressing the aorta should not be bulky nor endanger by its form or substance the adjacent parts; and it should admit of easy readjustment at subsequent operations, should they be indicated. Metal bands of silver and then of aluminium were employed with the hope that the amount of constriction might be regulated to a nicety at prospective subsequent operations as well as at the primary one. With the aid of an ingenious clock-maker, an instrument was devised to curl a metal strip, in perfect cylinder-form, about the vessel.

The tightening of the band is completed with the fingers; but in the early experiments, when the metal employed was too thick and the bands too broad, the aid of tweezers was required. We observed the first group of dogs with some apprehension, fearing that the edges of the band would cut the much constricted and powerfully pulsating aorta, and were considering ways to obviate, if necessary, this danger. On the twelfth day after operation a dog died of hemorrhage, the result of ulceration at the upper edge of the band. The experiments were thereupon discontinued for a while, to await results in the other dogs carrying aortic bands. Further cases of hemorrhage not occurring, we resumed, in a few weeks, the experiments. About three months later we investigated, at second operations and at autopsy, the resultant conditions and found that the

aortic wall where the band had embraced it was, in each instance of complete occlusion, atrophied, being reduced in some cases to a film-like thinness. Notwithstanding these somewhat discouraging observations the experiments were continued as actively as time permitted in the hope that, with an improved technique, derived from greater experience, particularly with reference to precision in the determination of the degree of closure brought about as the rolling (tightening) of the band proceeded, the walls of the arteries might retain their vitality even in the case of complete arterial occlusion (vide Cases 27 and 28). By reducing the width and length of the band and the thickness of the metal, we were able with the fingers, and with ease, to occlude the artery to the extent desired. From the first experiment we have endeavored, in each instance, to roll the band as perfectly (as cylindrically) as possible, flattening of it being assiduously guarded against. To control with accuracy the amount of blood-flow under the band, the rolling must go on smoothly and under the perfect command of the operator. The thrills of various strength, the point at which the pulse disappears, are carefully noted in the course of the act of constricting the vessel; and, finally, when complete occlusion is desired, the pulse being no longer distinguishable, the filling or not of the artery below the band, between it and a fine clamp placed central to the first distal branch is our only clue as to the patency of the artery.

With the use of silk or even silver wire such delicate manipulation and determination were found impossible, irrespective of the great danger of injury to the vessel-wall from attempts to draw just a shade tighter a half-knotted thread which has constricted the vessel almost to the occlusion point.

At the time of my first report (March, 1905)[4] we stated that: (1) If applied tightly enough to interrupt completely the circulation, the band had usually caused atrophy and frequently complete absorption of the aortic wall. In such cases hemorrhage was invariably prevented by the formation of connective tissue enclosing the band. (2) Thrombosis had not been observed in a single instance, either in the cases of complete occlusion in which the arterial wall under the band was found so greatly thinned, or in those in which it had

[4] *Loc. cit.*

been absorbed. (3) In a few cases (vide No. 28) aortic walls drawn together so snugly that at autopsy water could not be forced through with a syringe were, on division of the band, found to be normal and could be easily smoothed out and the full lumen reëstablished. But in these earlier cases of complete occlusion the aortic wall had almost invariably atrophied, having been so tightly constricted as to be deprived of its blood supply, and hence the "ideal obliteration" (reported the following year) by adhesion of the folded intima and the conversion of the constricted portion of the artery into a solid fibrous cylinder (vide Plate III, Fig. *2a*), could not have taken place. (4) The less snugly, the loosely and the very loosely applied bands might remain on the aorta, femorals and carotids for months without apparent injury to the walls of these vessels, either externally or internally. For example, the band after one hundred days would be seen shimmering brightly under a quite normal-looking peritoneum, having caused, as a rule, little if any reaction, and could be as easily removed form the wall of the artery as when originally applied. (5) We were encouraged to believe that there might be a place in surgery for the partially occluding band, and reported its application to the common carotid artery in the human subject and the manifestation, thereupon, of slight brain symptoms which persisted for several months. In this case the band was rolled more tightly than intended. It might easily have been removed and reapplied but, as I stated at the time, our notions being somewhat vague as to the precise amount of constriction to be desired and being unable to determine accurately the blood pressure distal to the band we decided not to disturb it and to note results. On the appearance of the head symptoms I did not relieve the constriction, believing that they would probably disappear; furthermore, I was not perfectly sure that, rather than good, harm might not result from the release of the carotid.

Subsequently (in June, 1906), in the report made in Boston before the Section on Surgery and Anatomy of the American Medical Association, the following newly observed facts were emphasized:

1. The blood pressure in the aorta below the band is lowered in proportion to the amount of the occlusion. The rise in pressure

below the band is, at first, rapid, but varies considerably in the different dogs. For example, in one dog, a rise below the band of ten millimeters (Hg. manometer) was noted in ten minutes, whereas, in another dog, two hours were required for a rise of fifteen millimeters. For the return of the normal pulse wave and of the normal blood pressure as many as seven months have been insufficient (vide history of Dog No. 96).[5]

2. Partially occluding bands produced as a rule no macroscopic alteration in the aortic wall under the band even after seven and eight months (vide Plate III, Fig. 1). Under completely occluding bands the arterial wall had (to the date of the second communication referred to) usually atrophied and in the course of weeks or months been absorbed. *"When the lumen had been, perhaps, not quite[6] occluded, complete obliteration might result spontaneously with the conversion of the arterial wall embraced by the band into a solid cylinder of living tissue. This may,"* I stated, *"be considered the ideal closure of an artery."* Although this form of arterial closure had occurred only thrice in the long series of experiments, it might, I thought, be accomplished frequently, and ultimately the band might be applied with such nicety that, unaided, further, by the surgeon, a partial would be likely to proceed to total occlusion.

3. *"The Effects on the Spinal Cord and Its Coverings.*—The study of the spinal cords was entrusted to Mr. P. K. Gilman (later Dr. Gilman and member of my staff), who discovered, in a number of instances,[7] about three months after operation, a deposit of extradural fat about the cord below the site of the aortic band. In three

[5] Since this statement was made we have had several opportunities to verify it, and have noted in a dog with partially occluding band on the thoracic aorta, after seven months, a difference of 30 millimeters or more in the pressure immediately above and immediately below the band. Of particular interest are the careful observations of Dr. Percy M. Dawson on the femoral and carotid pressures in Dog No. 96 of this series, reported below.

[6] We have not as yet learned to determine with the greatest precision the degree of arterial occlusion effected, nor am I convinced that in all of the cases in which the "ideal occlusion" has resulted (we have now five such observations) there may not have been, at the outset, complete obliteration of the lumen (vide history of Dog No. 27).

[7] These proved to be cases in which total or almost total occlusion had been made.

cases the production of fat was so great that it filled, seemingly under considerable tension, the vertebral canal."

If these observations should prove to be correct, Mr. Gilman has made a discovery of wide significance.

4. That in the human subject I had partially occluded the innominate artery once and the common carotid four times.[8] In the case of a large popliteal aneurism I had employed the metal band to occlude completely the femoral artery. In the case of a woman asphyxiated to unconsciousness by an aneurism of the aortic arch, I had exposed, carefully and freely and without puncturing either pleural cavity, the heart and arch of the aorta, hoping to be able to encircle with a band the aortic arch between the regions of the innominate and left carotid arteries, but the aneurism was so extensive and the patient's condition so desperate as to defeat the earnest and prolonged endeavor to execute the procedure.

THE ALUMINIUM BAND AND THE MANNER OF ITS APPLICATION TO THE ARTERY.

We have tested only two of the metals, silver and aluminium, with reference to their adaptability to the procedure under consideration. After a few experiments with the former it was discarded. The greater weight and value of the silver (there is great waste in its use) and the inconstant results obtained as to nicety in rolling, particularly after repeated boilings, are the principal factors which led to its disuse. The aluminium, usually purchased in sheets of about 25 degrees of thickness (American scale) should be cut, before being rolled down to the thinness desired, into strips of convenient length, and of width not greater than three quarters of an inch. If much wider, the strips warp inconveniently and have to be cut to waste in the selection of flat and regular parts for band material. It is well to stamp each strip with the numbers indicating the thickness of the metal and to have on hand a liberal supply of the various thicknesses from No. 25 to No. 46. The finest numbers

[8] The aluminium band has now been successfully applied in man to the common carotid artery twelve times, and once each to the thoracic aorta, the abdominal aorta, the common iliac, the femoral, and the innominate arteries. These cases will be reported later in the *Amer. Jour. of the Med. Sciences.*

we have used on the very small femoral and renal arteries of the dog. In the average dog for the abdominal aorta Nos. 34 to 35 are suitable, and for the thoracic aorta Nos. 33 and 34 we have used most frequently. In the human subject for the abdominal aorta below an aneurism near its bifurcation No. 33 sufficed, but ordinarily a heavier size would be required; for the common iliac No. 32 answered the purpose admirably; for the thoracic aorta Nos. 22 to 25 perhaps; for the common carotid we have almost invariably selected No. 33. The length of the band should be about that of the circumference of the full artery. The width varies from about 2 mm. for the renal arteries in the dog to about 1 cm. for the thoracic aorta and innominate artery in man. Fig. b, Plate XI, depicts a band suitable in width for the human carotid and for the average dog's abdominal aorta. The band in this illustration we should now regard as too long for its breadth by approximately one third. It is best to sterilize the aluminium only once. It may become too brittle for perfect rolling by repeated boilings. When rolled down on the artery enough almost to obliterate the pulse, a band of seemingly proper dimensions has rarely described more than two complete circles. The filing or "manicuring" of the band is of very great importance. It should be curved like a finger nail at the forward end and at the other cut precisely at right angles to its long axis. With a file the edges should be made perfectly smooth, but not sharp, and the rounded end symmetrical. A carefully filed band coils more easily both in the instrument and under the fingers, and, what is more important, is not likely to cut the artery. The aorta is the only vessel that I have seen cut itself on the band, and then, with the exception, perhaps, of two very young dogs or puppies, only when the band was badly filed or clumsily rolled, as in the early experiments, especially those in which forceps were employed to supplement the work of the fingers in tightening the too broad and too heavy bands at that time employed. Silk ligatures, even when occluding the aorta only partially, have in my experiments repeatedly cut entirely through the aorta, and without causing the death of the animal. They may leave in their wake various forms of diaphragm which more or less obstruct the lumen of this artery. I shall refer to a case of this kind depicted in Plate XII, Figs. 5 and 6.

The Band Curler.—The original instrument (vide Plate XI) had, we soon discovered, three major faults. It was (1) too broad at the arterial end, (2) the band lacked anterior support as it was being pressed onward by the driving blade, *a* (magnified *a'*), and (3) the latter did not always engage the former, owing to the fact that it was too springy and was insufficiently linked to its fellow on which it glides. To remedy the tendency for the band to buckle forward, one was compelled to support it with the finger during the process of curling. This was occasionally a difficult and usually an awkward performance.

Plate XI, Fig. 2 shows an improved and satisfactory band roller or curler. In the full length drawing the instrument is not loaded; in the abbreviated sketch the band projects from the end, half curled. The principal defects of the old instrument have been remedied in the new. Buckling of the band is prevented by the boxing; the driving-plate can not spring away from its fellow, and the width of the instrument has been sufficiently reduced to permit it to be passed freely between the closely given off branches of the abdominal and thoracic aortas. When the thinner bands are used it is sometimes necessary to give the faintest tip backwards to each of the two right-angled corners of the band to insure its engagement in the downward thrust of the piston or driving blade. After it has encircled the artery the band may, if its corners have been bent, be freshly squared with the scissors before being curled tighter by the fingers. This is not, however, necessary. The curler being armed with the carefully filed band of correct proportions the plunger is made to engage the band and to force its convex end into sight before the instrument is passed under the artery. In Plate XI, Fig. 1, the act of curling the band about the artery is shown, after the old manner, in its first stage. As the curling proceeds the instrument is gradually withdrawn. In arranging for the tightening of the band, its convex end should lie on the wall of the artery and be overlapped by the square end. With a very little practice one learns to avoid bending or flattening the band in the process of tightening it. The band should be long enough to encircle the artery in the expanded state of the latter and the metal should be sufficiently thick and wide to sustain the curl given it. If perfectly rolled the inside and outside

circles of the metal touch each other at all points of the surfaces of contact and, in consequence, the cohesion force is greatest. The artery should be raised from its bed by two tapes held far enough apart to leave uncovered sufficient free space on the artery for the occupancy of the band. Traction on the upper tape should be made to interrupt the blood current and thus to reduce the size of the vessel. A band curler should be selected (we have, at present, four sizes of this instrument) which might make the metal describe a circle smaller than the distended or full artery but a little larger than the empty one; then, with the return of the blood current, the artery expands and may fill the band quite snugly.

After complete occlusion of the abdominal aorta the femoral pulse does not, usually, return for weeks or even months. And after incomplete occlusion of the thoracic aorta (Dog 96) the femoral pulse may be hardly discernible after seven months. The anastomotic circulation takes place through the vasa vasorum as discovered and so beautifully depicted by Luigi Porta,[9] and by way of the internal mammary and epigastric arteries as especially emphasized by Kast.[10] We have repeatedly observed the great increase in the vascularity of the abdominal wall, particularly on splitting the recti muscles but also in making mid-line incisions at operations subsequent to the one at which the band was applied.

Dog No. 2.—Large, savage, collie-like dog. *Operation I.* Morphia and ether.[11] November 4, 1904. Silver band, partially occluding the vessel, applied to the abdominal aorta below (?) the inferior mesenteric artery. Radicles of thoracic duct not injured. Pulse in femorals easily countable at end of operation, though much reduced in volume.

November 6, 1904. Dog in good condition, rather dull, walks about without apparent weakness in hind legs. Tests such as running up stairs were not made.

November 10, 1904. Apparently perfectly well. Is quite savage and threatens to bite when cage is entered.

December 8, 1904. Vigorous femoral pulse but seemingly weaker than might be expected in so large a dog. Health has been perfect. *Operation II.* Morphia and ether. Complete occlusion of aorta about 1 cm. above the silver band by heavy black silk ligature. The silver band glistened through a very thin and apparently normal peritoneum. It had excited little or no irritation.

[9] Luigi Porta, Delle alterazioni patologiche delle arterie per la legatura e la torsione, Milan, 1845.

[10] Kast, *Deut. Zeit. für Chirurgie,* 1879, xii, 405.

[11] All the animals were anæsthetized in the same way preparatory to operation.

On coming out of ether the dog showed signs of unusual excitement. Respirations, 136 per minute. One hour after operation dog was able to climb a flight of stairs. The hind legs were, however, in spastic condition, flexed on the abdomen, at the knees and hips, and very much weaker than the fore legs.

December 10, 1904. Dog is dull. Hind legs are dragged in walking but are not completely paralyzed. Movements still spastic.

December 14, 1904. Is lively and seems quite well. Bladder and rectum function normally. Scratches himself with left hind leg without apparent weakness.

January 27, 1905. Femoral pulses not yet definitely palpable but the arteries have become quite full.

February 1, 1905. Femoral pulse faint and not countable.

February 20, 1905. Fair pulse, countable.

March 6, 1905. Found dead. Was not observed yesterday (Sunday). *Autopsy.*—A large flat cork causing intestinal obstruction is the cause of death. The silk ligature above the band has cut almost through the aorta, its track being apparently healed; part of the loop projects into a little cavity, with newly formed walls of connective tissue, adjacent to and in front of the wall of the aorta. A filmy substance, like decolorized blood clot, is adherent to the track taken by the ligature in its course through the artery. The aorta under the band is a little thinned. Its endothelial lining seems normal.

In the light of subsequent experience it seems probable that the thinning of the aorta may have been caused by the ligature applied so close above the band—by its interference with the circulation in the arterial wall.

Dog No. 6.—November 19, 1904. Black bitch, length 31 inches. *Operation I.* Closure of abdominal aorta with aluminium band 1 cm. in width.

The pulse pressure in the left femoral artery as recorded by an assistant:

Before operation	150 mm.
After abdomen was opened	130 mm.
Band incompletely tightened	80 mm.
Tightening of band completed	20 mm.
After abdominal manipulations were ended	25 mm.
After closure of abdomen	30 mm.

The operators could not feel the aortic pulse below the band after it was completely curled, hence I have doubt as to the accuracy of the three observations on pulse pressure in the femoral artery made after the final tightening of the band.

November 21, 1904. Femoral pulse doubtful. Dog ran easily on level and mounted the first steps of a flight of stairs without definite signs of weakness. Her hind legs gave out before the top steps were reached. Temperature fore-feet, 70°, hind feet, 66°, Fahrenheit.

November 22, 1904. Hind legs still weak. Sits down after slight exercise. Pulse in femorals doubtful. Surface temperature of hind-flanks, 95°; fore-flanks, 97°; pads of feet, hind 97°; front, 71°.

November 25, 1904. Temperature axilla, 98.5°; groin, 95.5°. Hind legs less weak. Dog in good spirits. *Operation II,* 11:30 A. M. Ligature of black silk

obliterating aorta applied below the band. 3 P. M., bladder not distended. Bitch has showed no excitement since operation. Temperature, axilla, 96°; groin, 93° F.

December 13, 1904. Slightly stiff in hind legs. Dog is dull and indisposed to walk much. We attributed the condition of the dog in part to one-half grain of morphia administered before operation.

December 14, 1904. Movements in hind legs greatly improved. Dog walks with ease but is a little depressed and sits down frequently.

December 19, 1904. Perfectly well and active.

December 28, 1904. Well and active.

January 7, 1905. Well.

January 12, 1905. Well.

January 19, 1905. Well and strong. Faint femoral pulse observed for the first time since the first operation performed two months ago.

January 23, 1905. Femoral pulse definite.

February 1, 1905. Femoral pulse countable but is still very small.

March 6, 1905. Dog well but quite thin. Killed with ether. Femoral and thoracic conditions normal. On palpating aorta a small nodule is felt at site of ligature. The band is found directly in line of the aorta above the ligature and filling completely a new-formed connective tissue cavity. The arterial wall under the band has been completely absorbed and the aortic wall above the band is continuous with the new connective tissue which forms a strong capsule about the band. The capsule of fibrous tissue fits the band so closely that no blood has escaped from the patulous aorta above into the space between the band and the connective tissue which enclosed it. The arterial lumen just below the band has been obliterated by the silk ligature which has cut entirely through the arterial wall and is enclosed in a hard nodule of connective tissue.

Dog 20.—Small puppy, male. December 10, 1904. 11 A. M. *Operation* Aluminium band applied above the inferior mesenteric artery. In exposing the artery the sigmoid flexure was pulled to the right. The band had been cut too long. It rolled beautifully to a certain point and then, before the aortic pulse could be obliterated the metal cylinder began to flatten. The pulse then became entirely obliterated but some sort of pulse-shock could be felt for a distance of 1 cm. or more below the band. 1.30 P. M., dog runs about, but his hind legs are very weak, though not completely paralyzed.

December 19, 1904. Hind legs stronger.

December 20, 1904. Dog does not seem well. Is quite thin and disinclined to walk. Can, however, stand and uses his hind legs in walking fairly well.

December 23, 1904. Not so well.

December 26, 1904. Quite ill.

December 27. Dead.

Autopsy.—The abdominal wound had opened down to the peritoneum but was well sealed by omentum and the subperitoneal flap of fat. There is no peritonitis. The portion of the ileum lying in the pelvis, and the sigmoid flexure exhibit punctate hemorrhages in the fat along the vessels. The sigmoid flexure is particularly hemorrhagic and probably the cause of the bloody fluid in the pelvis. There is some blood escaping from the anus. The jejunum, at a point near the aorta where it may well have been pressed upon by the retractors and gauze, is also indurated and dark. The bladder is empty. The band has excited very little reaction. The peritoneum and fat over it are still ununited.

Dog No. 27.—Small, black puppy. December 26, 1904. *Operation.* A very broad band (7 mm. in width) was placed on the aorta about 1 cm. below the inferior mesenteric artery and rolled by tweezers until the demonstration of a pulse below the band was questionable. We attempted in this case, prior to the application of the band, to produce occlusion by silver wire wrapped several times about the artery and over a small copper rod (the rod to be afterwards withdrawn), thinking that the size of the lumen might in such cases be definitely regulated by the use of rods of various sizes. After several trials we were convinced that the liability of injury to the artery was too great, at least much greater than with the employment of the band. The wrapping of the wire was also troublesome. Hence the project was abandoned.

December 28, 1904. Walks fairly well but has definite weakness in hind legs. Has fluid stools which contain a few drops of blood.

January 5, 1905. Weak and emaciated. A very small, not countable, femoral pulse has developed. Dog uses hind legs a little better.

January 23, 1905. Is very feeble and thin. Isolated in cage.

January 27, 1905. It is possible to-day for the first time to count the femoral pulse; it is still very small.

February 13, 1905. Condition practically unchanged.

February 20, 1905. Seems a little better. Femoral pulse still very small, but countable.

February 23, 1905. Improvement continues. Femoral artery feels fuller.

April 10, 1905. Dog is very thin and has the mange. Etherized. A very faint femoral pulse was demonstrable on dissection of the artery, but through the skin it was not definitely palpable. Dog killed.

Autopsy.—Peritoneal cavity normal.

The aortic band shimmers quite clearly through the peritoneum, but about 1 cm. below the band is a very delicate stellar cicatrix. The aorta was split to the band from both ends and the band itself divided. The vessel is entirely obliterated and the length of the obliterated portion corresponds exactly to the width of the band. The portion of the aorta enclosed by the band *has become converted into a solid fibrous cord* (vide Plate XII, Fig. 4*a*). Outside the band is a peculiar, soft, yellowish-white material, about one drop in quantity, resembling aleuronat. The hard, white, living cord, into which the occluded portion of the aorta has been converted, is 2 mm. in diameter. The band, an unusually broad and thick one, had been in place three and one-half months and might, without doing harm, have remained indefinitely. The form of the fibrous cord, so exactly cylindrical, under the band, is evidence that perfection in curling may be accomplished even with tweezers. Without especial effort to bring it about we have in five instances obtained this ideal

form of obliteration—four times in the aorta and once in the renal artery—and always in cases in which the completeness of the closure was in doubt at the time of operation. This case should be contrasted with Case No. 28, that of a small puppy whose aorta, though of intention completely closed by the band, was not found converted into a fibrous cord under the metal. To our surprise, it presented no internal adhesions whatever on being laid open, although so tightly constricted that not a drop of water could be forced through this portion of the vessel while the band was in place. The foldings of the aortic wall under the band could be smoothed out so completely that no trace of them remained, nor was there any abnormality of the wall to indicate that a band had been applied. We must bear in mind that in Dog 28 only twenty-two days had elapsed from operation to autopsy, whereas in the cases cited of fibrous cylinder formation, the dogs had carried their bands for several months.

Dog No. 28.—Small, black puppy, male. December 26, 1904. *Operation.* Aluminium band rolled (with tweezers) until pulse seemed to be obliterated.

December 28, 1904. Dog seems fairly well, but marked weakness and stiffness in hind legs.

January 7, 1905. Dog cross and disinclined to respond to attentions. Hind legs possibly a little less stiff.

January 12, 1905. Slight improvement noted.

January 17, 1905. Dog looks badly. Is greatly emaciated. Indisposed to move and hence the power in hind legs was not tested.

January 18, 1905. Great emaciation.

Autopsy.—Peritoneal cavity normal. Band shimmers through a perfectly normal-looking peritoneum. No trace of peritoneal or other scar over band. The healing about the band is the most absolutely perfect that we have as yet seen.

With a syringe connected to the aorta above the band we are unable to force water through the site of obstruction. On removing the band we find, to our surprise, that not only could the point of a scissors readily be passed through the constricted part but that the aortic wall under the band could be so perfectly smoothed out that not a trace of the foldings or wrinklings of the lining remained. Nor was there apparent the slightest thinning or alteration in the wall of the vessel at the site of the band. We have not as yet had the time to complete experiments undertaken with a view to ac-

counting for the difference in the condition of the aorta at the site of constriction in the cases (Nos. 27 and 28) referred to.

Dog. 30.—Medium-sized collie bitch. December 27, 1904. *Operation I.* Aluminium band tightened until the pulse was greatly reduced but not apparently obliterated. There was sufficient pulse just prior to the final tightening to give a faint thrill.

December 28, 1904. Dog convalescing normally. Uses hind legs quite well. No definite sign of weakness in them.

January 7, 1905. No weakness in hind legs.

January 19, 1905. Dog very well. Femoral pulse not yet distinguishable.

January 23, 1905. *Operation II.* 11 A. M. There were a few adhesions of omentum in the neighborhood of the band, which prevented the metal from shimmering in the usual manner through the peritoneum. Pulse below the band is feeble but perfectly definite. When the pulse just above the band is obliterated with the finger the pulse below the band disappears. We concluded consequently that the band had not completely closed the lumen of the aorta. Ligation with heavy black silk 3 or 4 mm. above band. Much to our astonishment the pulse, easily appreciable by finger, reappeared below the band in less than 30 seconds after the aorta was ligated. It seems almost inconceivable that a ligature applied so close above the band should have had such a marked influence on the pulse below it. We have made this observation in a number of cases in which we were quite sure that no vessel of a size to be appreciated was given off from the aorta between the ligature and the band.

The almost immediate reappearance of the pulse after the application of the ligature indicates that the anastomotic circulation was already quite well established, for a pulse large enough to be appreciated by the finger has not been observed by us before the termination of the operation, after complete closure of the aorta in one act. We have, however, noted that within a minute after ligation of the abdominal aorta a faint pulse below the ligature can be *seen* (not felt), provided the aorta is cut open.

January 23, 1905. 3 P. M., Dog runs about. Weakness of hind legs not apparent.

January 24, 1905. A very faint femoral pulse can be felt very high up on the femoral arteries. No weakness of hind legs.

January 27, 1905. Pulse more easily felt but still feeble.

February 1, 1905. Femoral pulse can be counted. The artery is not full.

February 20, 1905. Artery much fuller. Pulse stronger. Dog quite thin.

March 10, 1905. Femoral pulse still small. Dog is thin but looks well. Ether administered. Abdomen opened. Small indurated area about the ligature and the band. A definite pulse below the band is promptly shut off by pressure immediately above the band and at higher points, as noted in history of Dog No. 6. A ligature was applied about 3 cm. above the band and tests for a pulse below

the band were made for three minutes but it had not returned in this period of time. The dog was then killed. Above the ligature the aorta was entirely occluded in a conical manner. The ligature lies in a small cavity lined with granulations. Below the band the aorta is closed off by a small transparent film. The band lies in a connective tissue cavity and contains merely a film of opaque secretion. The aortic wall under the band has consequently been entirely absorbed. Between the two cavities a piece of aorta 13 mm. in length is felt with a partly organized clot which is translucent except for a red dot about 1 mm. broad, in its center.

It is evident that at the first operation I was probably mistaken in believing that the artery was not obliterated by the constricting band. It would, a priori, seem reasonable to presume that a band though not altogether occluding the artery, might, nevertheless, so greatly interfere with the circulation of its wall as to lead to necrosis, but the findings in Dog. No. 28 would seem to make such a view untenable. It seems, however, not unlikely that the necrosis of the wall of the aorta may not have supervened until at the second operation the ligation with silk a few millimeters above the band was made. If this were the case it may be that our impression that the pulse had not been obliterated by the band at the first operation was correct.

Thoracic Aorta Partially Occluded by Aluminium Band.—Dog No. 96.—Medium-sized Newfoundland bitch. May 22, 1906. *Operation.* Assisted by Drs. Richardson and Gilman. Lungs inflated during the operation through tracheotomy tube. Incision through seventh interspace. Ribs separated as usual with self-retaining retractor. Exposure of aorta very satisfactory. Aluminium band, thickness No. 32, width 6 mm., applied just above highest point of diaphragm. Band was tightened until a continuous thrill below the band was produced.

May 24, 1906. Slight stiffness in hind legs.

May 25, 1906. Improving; takes milk. Femoral pulse doubtful.

May 27, 1906. Cheerful; runs about freely. Percussion reveals no thoracic signs. Chest not auscultated.

June 1, 1906. Dog is lively and well.

June 3, 1906. I went to Boston.

June 11, 1906. On my return from Boston to-day dog was reported well. I did not visit her.

June 14, 1906. On visiting dog surprised to find that she is emaciated, low spirited and very weak. Lies in corner of kennel unless aroused. The keeper

states that, suddenly, two nights ago the dog became paralyzed. He assured me that she was perfectly well the preceding day. Has made no observations as to her stools, urine or feeding. I placed her at once in a separate room for observation. Find that she can raise herself on hind legs and totter about with hind legs stiff and held apart. Power in hind legs quickly becomes completely exhausted.

June 15, 1906. Better. Takes milk and eats a little.

June 16, 1906. Improving. Has some appetite and a stronger gait. The femoral artery is fairly full but a pulse is not palpable.

June 18, 1906. Stronger and in fairly good spirits. Is as lively as hind legs permit but still has difficulty in getting in a standing position; but when balance has been obtained runs about quite well, but for a few moments only, the hind legs weakening rapidly, then a few steps alternating with dragging. Femoral arteries full and quite tense but no pulse is as yet discoverable although carefully palpated for by several observers.

June 19, 1906. At times there seemed to me to be a faint pulse in the femoral artery. Dog much livelier, eats better and gait is improved. She still experiences considerable difficulty in raising herself upon her hind legs, and after running five or six seconds flops down behind. She is up again immediately and again collapses as to hind legs. At no time have the stools been bloody or tarry. There has been no evidence either of retention of urine or difficulty in micturition. The dog is to be kept all summer under careful observation.

December 21, 1906. Since the last note the dog has been well but is still weak in the hind legs. No observations of especial interest were made during the summer. Careful examination by Dr. Sowers, Watts and myself determine that a femoral pulse is present but not easily countable, although it is now seven months since the operation. Only the lightest pressure of the fingers can detect this femoral pulse. Ether is administered and the dog brought into the surgical amphitheatre for demonstration to the class. The over-pressure box of Drs. Follis and Fisher was successfully employed throughout the observations which follow. On opening the chest the left lung and pleura were normal. The band is seen shimmering clearly through the thin and normal-looking pleura covering it. A very pronounced thrill is felt below the band on the thoracic aorta, just as at the termination of the first operation, hence our belief that the artery had suddenly closed about three weeks after the first operation and during my absence in Boston is proved to be incorrect. Dr. Percy M. Dawson then made the following observations upon the blood pressure in the carotid and femoral arteries.

"Upon the circulation of this animal observations were made in the physiologic laboratory and were reported as follows:

	Maximum Pressure.	Mean Pressure.	Minimum Pressure.	Pulse Pressure.
Femoral	116	96	88	28
Carotid	160	113	83	77

These figures should be compared with values obtained in normal animals,[12] namely,

Femoral	188	120	95	93
Carotid	162	122	103	49

"The pressure curves were also obtained and showed a total disappearance of the dicrotic elevation from the femoral pulse whereas in normal animals the dicrotic is more marked in the femoral than in the carotid artery so that hyperdicrotism is more readily obtained in the former than in the latter.[13]

"All these changes are to be attributed to the constriction due to the band and are in complete accord with the unpublished experiments performed in this laboratory in which the aorta was partially occluded for the purpose of studying the relation of the carotid and femoral pulses.

"Considerable variations occur in the velocity of the pulse wave in different dogs. Nevertheless it is safe to say that a velocity of only 556 cm. per second as was found in this animal, owes its small value to the aortic constriction."

Dr. Richardson and I, by the Huertle method with hypodermic needle, observed, much to our surprise, that whereas the manometer registered 126 mm. above the band, it registered only 94 below it. *It seems remarkable that after so long a period (approximately seven months) there should be such a difference in the blood pressure above and below the band.* Just before death the aorta was completely divided below the band to determine the degree of patency of this vessel at the constricted point. The heart pumped through the band a small stream as large, perhaps, as that furnished by the human radial artery.

[12] *Amer. Jour. of Physiol.*, 1906, xv, 244.
[13] *Amer. Med.*, 1906, i, 152.

Autopsy.—There is a very great difference in the size and thickness of the thoracic aorta above and below the band. The wall of the artery above the band appears to be twice as thick and much stiffer than the arterial wall below it. There is also great dilation of the artery above the band. There are no visible calcareous plaques. On cutting through the artery and band in the usual way, the probe point of a scissors being passed into the lumen of the vessel, there is found to be no adhesion between the folded surfaces although, until the artery is spread quite flat, the foldings and creasings of the intima are evident. Were it not for the presence of the band which reveals its form from within by pressure on the wall of the artery from without it might be difficult if not impossible to determine with the naked eye differences so far as the free surface of the intima is concerned, below and beneath the band. The lining of the aorta under the metal seems perfectly normal, as indeed does the entire thickness of the arterial wall. The spinal cord was excised by Dr. Gilman in my presence. As to the extradural fat formation in this case a report will be made later.

EXPLANATION OF PLATES.

Plate XI.

Fig. 1. Drawn in 1905. The original band roller in the act of curling a metal strip about an artery; *a*, the tip of the driving blade enlarged; *b*, the metal strip; *c*, the band slightly tightened with the fingers as when a degree of incomplete occlusion is desired. The proportions depicted are those observed at the time the drawing was made. We should now regard the length of the metal strip as about one-third too great for its width as well as for the size of the artery represented.

Fig. 2. The improved band roller—the size usually employed in the experimental work. The instrument shown in full length is unloaded. In the abbreviated cut the band is about to be expelled from the roller. This band is broad enough for the abdominal aorta in man, and the diameter of the circle is too short for a vessel requiring such a broad band.

Plate XII.

Fig. 3. Aorta of dog after partial occlusion by band for one month. The band (*b*), in outline, is seen through the vessel's wall. Fig. 4. Aorta of Dog 27 converted into a solid cylinder (*a*), the band (*b*) having embraced the artery for three and one-half months.

Figs. 5 and 6. An aorta about which a partially occluding ligature of silk had been placed. In Fig. 5, the wall of the artery is divided anteriorly only enough

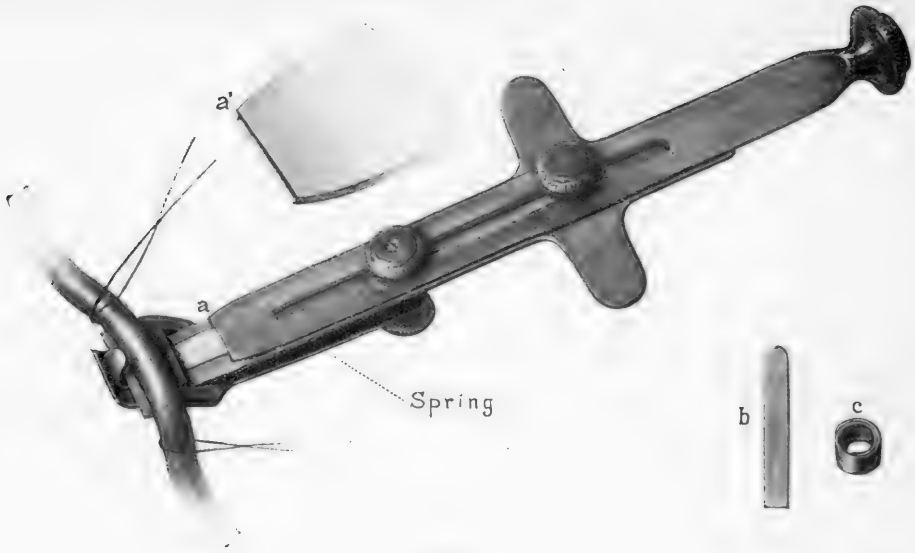

FIG. 1.

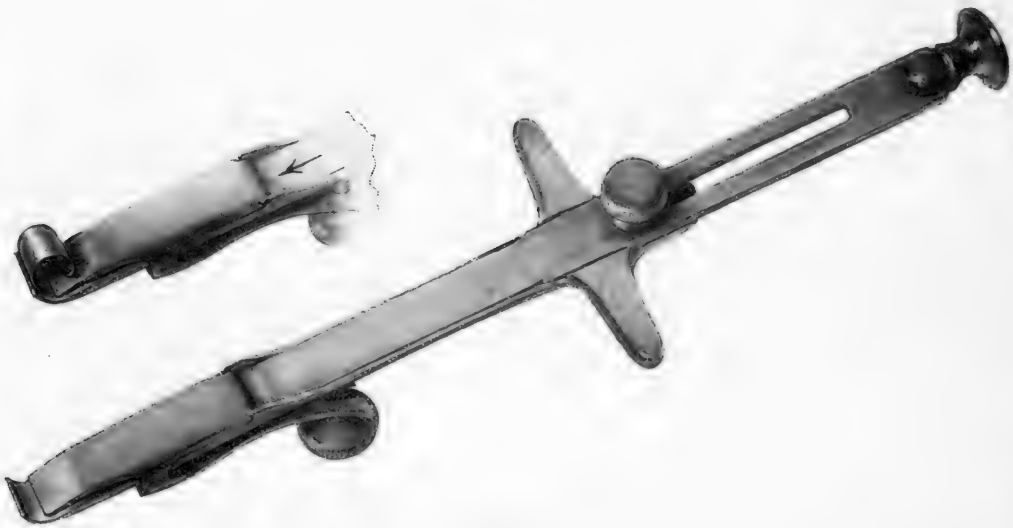

FIG. 2.

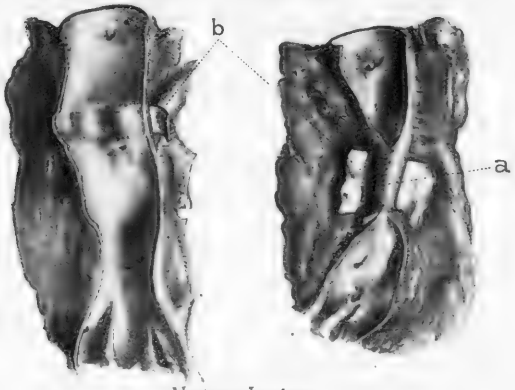

FIG. 3. FIG. 4.

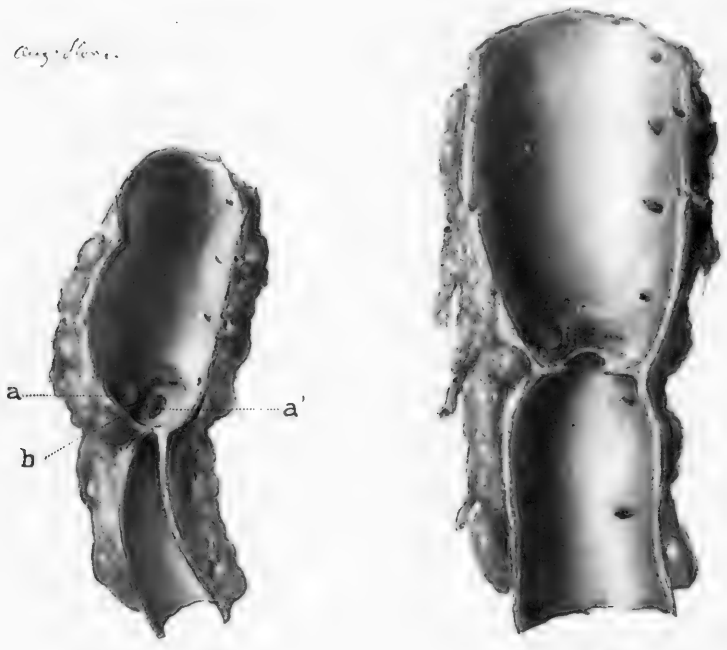

FIG. 5. FIG. 6.

FIG. 7.

to expose the diaphragm formed in the track of the ligature and perforated by two holes, *a* and *a'*. The silk ligature, at *b*, is more plainly seen in Fig. 6, in which the diaphragm has been divided as far as and into the anterior perforation.

PLATE XIII.

FIG. 7. The kidneys of a dog six months after the application of an occluding band to the renal artery of one of them. The band, spread out, lies *in situ* under the right renal artery which had become converted into a solid cylinder at this point, but was collapsed both proximal and distal to the occluding metal. Contrast the renal arteries of the two sides.

A NEW AND SIMPLE METHOD FOR THE SERUM DIAGNOSIS OF SYPHILIS.[1]

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Since the important discovery by Wassermann of serodiagnosis of syphilis, an enormous amount of work has been done, and to-day its value as a diagnostic test is beyond dispute. The complexity of the original method and the requirement for a thorough knowledge of hæmolysis and skill in serological technique have, however, been a great obstacle to its introduction into general practice. I am convinced that, unless a great simplification of the method of the Wassermann test can be devised, it will never attain the usefulness which it really deserves. Many workers have attempted to simplify the method, but thus far, according to my view, without success.[2]

[1] Received for publication, January 15, 1909.

[2] Judging from my own observations which extended over 100 cases, Bauer's method (*Deut. med. Woch.*, 1908, xxxiv, 698) is not only unreliable on account of the inconstant content in natural anti-sheep amboceptor in human serum, but it is also inconvenient, because it requires washed sheep's blood corpuscles and the special apparatus of a laboratory. The method of Detre and v. Brezovsky (*Wien. klin. Woch.*, 1908, xxi, 1700–1743) appears about equal in reliability to the original, but in spite of its ingenious technique the use of washed horse corpuscles interferes with its usefulness outside of a laboratory.

A so-called simple method of serum diagnosis of syphilis recently published by Tschernogubow (*Berl. klin. Woch.*, 1908, xlv, 2107) is of a different order since he states that the complement contained in the blood of patients is sufficient in quantity to reactivate anti-human amboceptor and cause hæmolysis of the same blood. No mention is made of the source and strength of the anti-human amboceptor referred to. Tschernogubow's method relies upon an unknown quantity of complement which varies in different specimens of human blood. As the complement and erythrocytes deteriorate rapidly, his method is inapplicable to old specimens. There is also no direct way of testing the inhibitory action of the antigen alone. Anti-human amboceptor derived from immunized rabbits is reactivale only by large amounts of human serum, which renders the method inconvenient. Finally the idea of Hecht (*Wien. klin. Woch.*,

All the methods so far proposed are indeed suited only for the fully equipped laboratory possessing centrifuge, thermostat, water-bath, etc.

I have, I believe, been successful in overcoming the difficulties of the situation by perfecting a new and simple method of syphilis serodiagnosis. This method offers definite advantage in point of delicacy and simplicity for the experimental serologist; and the reagents to be employed can be prepared for and put within reach of the practitioner so that the test may be made by any physician who is in the habit of making clinical laboratory tests, such as the Widal test, hæmoglobin estimation, etc.

PRINCIPLES OF THE METHOD.

The new method is based upon the same principle as Wassermann's and utilizes the Bordet-Gengou phenomenon of complement-fixation to determine the presence of the syphilis antibody in a given specimen of blood serum or cerebrospinal fluid. It differs, however, from the Wassermann method in using an anti-human hæmolytic system instead of an anti-sheep hæmolytic system. This difference may at first seem of trifling importance but it really is very significant since it affects the accuracy, ease and reliability with which the test can be applied. Wassermann's original method, it may be added for sake of clarity, is subject to an error arising from the presence in human serum of a varying amount of natural amboceptor capable of being reactivated by guinea-pig's complement. Many specimens of human serum contain in 0.1 c.c., which is the quantity usually employed in a test for complement fixation, as many as twenty units of anti-sheep amboceptor, while some specimens are entirely devoid of this amboceptor. I found by actual experiment that four units, but not two units, of the natural anti-sheep amboceptor prevent entirely the detection of one unit of syphilis antibody. When eight units of natural anti-sheep amboceptor and two units of syphilis antibody are brought together,

1908, xxi, 1742) that the native hæmolytic property of human serum alone may be used as an hæmolytic system in combination with washed sheep's corpuscles is impracticable, as the amount of human complement and amboceptor is so variable as to make the test unreliable without a special standardization of such serum.

the test remains completely negative. In other words, by the method of Wassermann the presence of syphilis antibody may be missed although in the absence of the natural anti-sheep amboceptor it would be readily detectable. This defect in the complement fixation system is not only inherent to the Wassermann method but also to all those which employ foreign blood corpuscles for which human serum contains natural hæmolytic amboceptors capable of being reactivated by the complement employed in the test.

I believe that the method which I have worked out eliminates completely this source of error, because human blood corpuscles are used as the hæmolytic indicator in combination with human serum, so that as no foreign natural hæmolytic amboceptor is accidentally present, its effects are excluded. The present method of the complement fixation test is carried out under conditions of definite and uniform sensitization of the blood corpuscles, thus enabling one to detect the presence of even a fractional part of one unit of syphilis antibody in a given specimen. It is impossible to miss one or more units of the antibody, which happens not infrequently with the Wassermann and similar methods.

DESCRIPTION OF THE METHOD.

In order to carry out the present method of diagnosis it is needless to say that the serum of the patient under question must be obtained. The patient's serum is then subjected to the usual complement fixation test with the reagent to be described immediately below and according to the technique specially adapted for the present method. I have developed so far two different ways of applying the test. In one the reagents are employed in liquid form and in the other, the reagents are used in a dried state on filter paper. The first way of making the test is adapted to general biological laboratories, and the second is adapted especially for the clinical laboratory. The descriptions which follow immediately refer to the laboratory method, but they do not differ essentially from those for the clinical laboratory except that the reagents employed are in the two different forms mentioned.

REAGENTS REQUIRED FOR THE METHOD.

The following reagents are required for making a test by the present method:

1. *Anti-human hæmolytic amboceptor* prepared in rabbits by injecting them five or six times into the peritoneal cavity with increasing doses (up to 20 c.c.) of washed human blood corpuscles, allowing five days interval between each injection. The serum is collected from the immunized animal eight or nine days after the last injection. The titre must be stronger than 0.01 c.c. for complete hæmolysis.[3] I now use a serum of 0.001 c.c. titre.

2. *Complement.* Fresh guinea-pig serum.

3. *Antigen.* Alcoholic extract of organs or crude preparations of lecithin. To prepare the alcoholic extract of organs one part of mashed tissue (liver, kidney) is extracted with ten parts of absolute alcohol for several days at 37° C., filtered through paper and the filtrate concentrated to about one-third of its volume, and the fluid preserved. To prepare the lecithin solution for antigen, 0.3 gram is dissolved in 50 c.c. of absolute alcohol and then shaken with 50 c.c. of physiological salt solution and filtered. The filtrate must be clear.

4. *Suspension of human blood corpuscles.* This is prepared by mixing one drop of the blood of a normal person[4] with 4 c.c. of physiological salt solution.

5. *The serum to be tested.* About ten drops of blood from a patient are collected in a small test tube. The clear serum separated from the clot is employed for the test.

METHOD OF MAKING THE TEST.

1. Take six clean test tubes (size 10 cm. x 1 cm.). In the first two of these, place one drop from a capillary pipette (or tube if necessary) of the patient's serum which is to be tested. In each of the second two tubes (which are to serve as controls) put one drop of the serum of a syphilitic case known to give the positive

[3] Complete hæmolysis of 1 c.c. of human blood suspension (¼ drop) in the presence of 0.025 c.c. of guinea-pig's fresh serum.

[4] The suspension can also be prepared with the patient's blood, but it must be used only in combination with the serum of the same patient.

reaction. In each of the third pair of tubes (also controls) put one drop of the serum of a normal person. Now to each of the six tubes add 1 c.c. of the suspension of human blood corpuscles and 0.04 c.c. of fresh guinea-pig serum as complement. Lastly into one of each of the three foregoing pairs of tubes put one drop of the antigen solution from a capillary pipette. The second tube of each pair receives no antigen.

2. After being well mixed by shaking, the six tubes are incubated at 37° C. for one hour.

3. At the end of the incubation, add two units of anti-human amboceptor to each tube and mix well by shaking. Incubate the tubes for two hours longer at 37° C. Read the reaction from time to time for the next ten to twelve hours during which the tubes are kept at room temperature.

The complete test is shown in the following chart.

CHART I.

	Test for Diagnosis.	1st Control Set.* Test with a Known Syphilitic Serum (Positive Reaction).	2d Control Set.† Test with a Normal Serum (Negative Reaction).
Control tube without antigen for each test.	*a*. Patient's serum. ○ *b*. Human blood suspension. *c*. Complement.	*a'*. Positive luetic serum. ○ *b*. Human blood suspension. *c*. Complement.	*a''*. Normal serum. ○ *b*. Human blood suspension. *c*. Complement.
Determinative tube containing antigen.	*a*. ○ *b*. } ditto. *c*. *d*. Antigen.	*a'*. ○ *b*. } ditto. *c*. *d*. Antigen.	*a''*. ○ *b*. } ditto. *c*. *d*. Antigen.
Incubation at 37° C. for 1 hour.			
Anti-human hæmolytic amboceptor to all tubes.			
Incubation at 37° C. for two hours longer, then at room temp.			

* When many specimens of syphilitic sera are subjected to simultaneous tests this set of controls is scarcely necessary, as we usually find a good positive serum among them.

† This control set may be made without the addition of any human serum.

It is necessary to begin the test with one drop of the patient's serum. If the reaction is negative the test should be repeated with two drops. Sera are not infrequently met with, however, in which two drops are inhibitory without the presence of antigen. On this

account it is undesirable to use two drops as routine. Usually one drop reveals the presence of the specific antibody if it is present at all, and further test becomes almost unnecessary.

READING OF THE REACTION.

A negative reaction is indicated by complete hæmolysis in both tubes irrespective of the presence of the antigen. On the other hand, a positive reaction is shown by complete absence or partial inhibition of hæmolysis in the tube containing the antigen and complete hæmolysis in the tube without the antigen.

Complete hæmolysis in the negative control takes place usually within one hour or sometimes earlier. When the reaction is strongly positive, as it should be with the positive control set, the tube with the antigen remains absolutely free from any trace of hæmolysis even after a few days' standing at room temperature. In such a case the erythrocytes are strongly agglutinated and remain adherent to the bottom of the test-tube, leaving above a perfectly clear medium. In moderately positive cases there is a trace of hæmolysis, while the varying degree of positive reaction can readily be measured by the amount of the hæmoglobin liberated in the medium and especially by the amount of intact red corpuscles remaining in the fibrin shreds or whitish stroma masses. It should be mentioned that the tubes containing the antigen undergo hæmolysis somewhat more slowly than those without antigen, but complete hæmolysis must occur in both tubes of the negative control set, before the reaction of the other sets of tests is read. It is also necessary to note that every tube without antigen, irrespective of the nature of the specimen of blood serum therein contained, must undergo complete, or at least almost, complete hæmolysis before the final reading of reaction is made. In cases in which tubes without antigen do not undergo hæmolysis the test must be repeated with a smaller quantity of the human serum in order to obtain a definite result.

SIMPLIFIED METHOD FOR THE CLINICAL LABORATORY.

The method so far described is much simpler than the original test of Wassermann and can be quickly and easily carried out in any

laboratory where experimental work with serum is constantly being conducted. I have, moreover, been able so to modify the methods of handling the various factors in the test that it should be possible to carry it out with accuracy and dispatch in any clinical laboratory. These further modifications consist essentially in drying the reagents on paper and standardizing them. In this form they can be prepared on a large scale by commercial biological laboratories under supervision of a competent serologist, and placed on the market within ready reach of physicians. My experiments have shown that it is possible to do this with great accuracy and that the preparations so made are stable when kept under ordinary conditions. In the following paragraphs I will describe the method of preparing the filter paper reagents and the mode of employing them for the test.

Anti-human amboceptor slips. The serum of immune rabbits is taken up with filter paper and quickly dried (a few hours) by a current of air at low temperature (below 20° C.). If the immune serum is not very strong—the titre 0.01 c.c. for example—the serum should first be concentrated to one-third of its volume by means of a current of air before the paper is impregnated. This is necessary in order to secure a slip of small dimension for convenient use.) After complete desiccation, the impregnated paper is cut into pieces of equal dimensions, each one being of such a size as to contain two units of the amboceptor.

Complement slips. A rather thick filter paper is impregnated with fresh guinea-pig serum, similarly dried and cut into pieces of equal size. The activity of the complement slip must be titrated and compared with the fresh complement. The activity of the slip-complement may vary somewhat with every preparation, but one slip should possess such an activity as to correspond with that of 0.04 c.c. of the fresh guinea-pig serum.

Antigen slips. A crude preparation of lecithin of previously known antigenic value is dissolved in ether and similarly taken up into filter paper. It is also easy to impregnate the paper with the alcoholic organ extracts (such as from the liver of a congenitally syphilitic fœtus), but a preliminary concentration of the extract by means of a current of air is necessary in this case. After impreg-

nation and complete desiccation, the impregnated paper is cut into equal parts, each being of such a size as to contain enough antigen for one tube.

These three reagents, the anti-human hæmolytic amboceptor, guinea-pig complement and antigen, in the impregnated filter papers, can be kept indefinitely at room temperature in a dry place. I have adopted as the dimension of each slip the size of about 5 mm. by 5 mm. It is understood that preliminary tests should always be made with the slips to prove activity and strength.

In employing the filter paper slips, they are dropped by means of forceps into the test tubes already containing the human blood suspension and patient's serum, in the order and at the intervals already stated for the respective reagents (complement, antigen, amboceptor) in the description for the method of making the test (Chart I). It is necessary to shake the tubes a few times at intervals in order to insure proper solution of the reagents. The incubation may be carried out perfectly well in the vest pocket if a thermostat is not at hand. Confusion is to be avoided by carefully labelling the various tubes. Each of the pairs of tubes making up, respectively, the test, and the two control sets, may conveniently be held together by a rubber band. There can be no confusion within one pair of tests since one of the two tubes contains one more slip (antigen) than the other.

I should advise any one contemplating the use of the method to make a few preliminary trials comparing cases definitely syphilitic with normal individuals before undertaking the diagnosis of cases of unknown nature. This would also have the advantage of accustoming the physician to the order of the procedures and the reactions and serves as a test of the reliability of the reagents.

RESULTS.

In all, four hundred and sixty-five specimens of serum have been examined by the method above outlined. One hundred and fifteen of these were examined at the same time by the original method of Wassermann. The results are tabulated as follows:

TABLE I.[5]

Amount of Treatment.	*O*				Slight.				Moderate.				Fairly Well.				Well.			
Method.*	*W*		*N*		*W*		*N*		*W*		*N*		*W*		*N*		*W*		*N*	
Reaction.	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—
Primary Lues.	4	2	6	0	1	0	1	0												
Secondary Lues.																				
Manifest.	18	2	20	0	4	0	4	0	1	2	3	0								
Latent.	1	0	1	0	0	1	1	0	4	2	6	0	1	1	1	1	0	2	0	2
Tertiary Lues.																				
Manifest.	3	0	3	0	4	0	4	0	8	0	8	0					2	1	2	1
Latent.									5	2	6	1	1	1	1	1	5	4	7	2
Suspects.	16	12	21	7																
Non-syphilitic.	0	5	0	5																

* *W* = Wassermann's test; *N* = the present test.

Summarized the results are as follows:

Of 7 cases of primary syphilis the Wassermann test was positive in 5, the present test in all.

Of 27 cases of manifest secondary syphilis the Wassermann test was positive in 23, the present method in all 27.

Of 12 cases of latent secondary syphilis the Wassermann test was positive in 6, the present method in 9.

Of 18 cases of manifest tertiary syphilis the two methods agreed and were positive in 17.

Of 18 cases of latent tertiary syphilis the Wassermann test was positive in 11, the present method in 14.

Of 11 untabulated cases of tabes dorsalis only 3 were positive with the Wassermann test and all were positive with the present test.

Of 28 cases suspected of syphilis or of syphilitic origin, the Wassermann test was positive in 16, the present method in 21.

Concerning certain points in the manner of employing the various reagents in testing the cases above reported, I may state that about one-third of all the cases were examined with liquid preparations

[5] The cases recorded in this table were derived chiefly from the clinics of Dr. Victor C. Pedersen of the New York Hospital and House of Relief, Dr. Howard Fox of the Vanderbilt Clinic, and Dr. J. A. Fordyce (through Dr. Homer F. Swift) of Bellevue Hospital. Ten of the suspected cases belong to Dr. Jacob Heckmann of the German Hospital Dispensary, and will be reported by him from a clinical standpoint in a later publication. It is my pleasant duty to acknowledge my indebtedness to these gentlemen for their assistance in furnishing me with the clinical material.

of complement, antigen and amboceptor, while a second third were tested in a similar manner except that the complement and amboceptor were first dried and pulverized before they were dissolved in saline solution for use. The remaining third of the cases were examined by the reagent paper method. The results obtained by this last method were perfectly reliable and satisfactory. It is needless to say that every one of these tests had been carefully controlled by the use of liquid reagents and by the regular Wassermann test.

In comparing the results obtained by the original Wassermann method with those obtained from the simple method here described. it should be pointed out that cases which were negative to the Wassermann and weakly or often quite strongly positive to the present method were met with. The causes of the observed differences were found in the existence of a large amount of natural amboceptor for sheep's blood corpuscles in such specimens of human serum. No disagreement in the result between the two methods was found with strongly positive specimens (containing more than eight units of syphilis antibody in 0.1 c.c.) or with weak specimens devoid of the natural amboceptor.

I would finally draw attention to the value of the test as a guide to the treatment of cases of syphilis. It appears that with the progress of the cure the symptoms of the disease not only abate, but the blood reaction grows weaker until ultimately when the cure has been established the reaction can no longer be obtained. If experience supports this result, the duration of the antisyphilitic treatment and its efficacy may become subject to quantitative control. The advantages which would accrue from such a measure are obvious. I mean to pay strict attention to this important therapeutic point and shall hope to report on a much larger series of cases in the near future.